Volume _____

FINAL REPORT

Virucidal Efficacy Test for A Test Device- Middle East Respiratory Syndrome Coronavirus (MERS-CoV)

Test Substance
STR-Solution

Test Organism
Middle East Respiratory Syndrome Coronavirus, BEI Resources

Author
Cory Chiossone

Study Completion Date
6/30/16

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

Laboratory Project Identification Number
922-101

Protocol Identification Number
FOR.1a.04.28.16

Sponsor
Forward Medi Co,. Ltd.
HangGang Xi Tower B-dong
Room 804, 1498 Gayang-dong
Gangseo-Gu, Seoul, Korea

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COMPLIANCE STATEMENT

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

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

The following technical personnel participated in this study:

Cory Chiossone, Jessica Wagner, Semhar Fanuel

Study Director: MicroBioTest

Cory Chiossone 6/30/16
Date

QUALITY ASSURANCE UNIT STATEMENT

Title of Study: Virucidal Efficacy Test for A Test Device- Middle East Respiratory Syndrome Coronavirus (MERS-CoV)

The Quality Assurance Unit of MicroBioTest has inspected the Project Number 922-101 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	05/26/16	05/27/16	05/27/16
In Process	05/27/16	05/27/16	05/27/16
Final Report	06/29/16	06/29/16	06/29/16

Jeanne M. Anderegg, RQAP-GLP
Quality Assurance Manager
06-30-2016
Date

TEST SUMMARY

Title: Virucidal Efficacy Test for A Test Device- Middle East Respiratory Syndrome Coronavirus (MERS-CoV)

Study design: This study was performed according to the signed protocol and project sheet(s) issued by the Study Director (See Appendix).

Test substance(s) supplied by the sponsor of the study:

1. STR-Solution, received at MicroBioTest on 05/13/16, and assigned DS No. G282

Sponsor: Forward Medi Co,. Ltd.
HangGang Xi Tower B-dong
Room 804, 1498 Gayang-dong
Gangseo-Gu, Seoul, Korea

TEST CONDITIONS

Challenge virus:

Middle East Respiratory Syndrome Coronavirus (MERS-CoV), BEI Resources

Host:

Vero E6 cells, ATCC CRL-1586

Active Ingredients:

Adhvanced PCO

Dilution medium/Neutralizer:

Minimum Essential Medium (MEM) + 2% Fetal Bovine Serum (FBS)

Contact time:

1 hour, 2 hours, 4 hours

Contact temperature:

Ambient 20±1°C (Actual: 20°C);

Carrier inoculation and dry time:

A glass carrier was inoculated with 0.2 mL of virus in a 4 in^2 area and dried for 18 minutes at 20°C.

Exposure Distance:

Approximately 5 cm

TEST CONDITIONS (continued)

Test device preparation:

The test device was assembled and operated safely according to the manufacturer or Sponsor's instructions.

Organic load:

5% Serum

Media and reagents:

MEM + 2% FBS
MEM + 10% FBS
FBS
MEM

STUDY DATES AND FACILITIES

The laboratory phase of this test was performed at MicroBioTest, 105 Carpenter Drive, Sterling, VA 20164, from 05/26/16 to 06/03/16. The study director signed the protocol on 05/26/16. The study completion date is the date the study director signed the final report. The individual test dates are as follows:

- Testing started at 12:40 pm on 05/26/16 and ended at 4:40 pm on 06/03/16

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 substance 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.

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

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

RESULTS

Results are presented in Tables 1 – 3.

The Log_{10} Reduction Factor was calculated in the following manner:

Log_{10} Reduction Factor = Initial Viral Load (Log_{10} $TCID_{50}$) – Output Viral Load (Log_{10} $TCID_{50}$)

The Viral Load (Log_{10} $TCID_{50}$) per carrier was calculated in the following manner:

Viral Load (Log_{10} $TCID_{50}$) = Titer (Log_{10} $TCID_{50}$/mL) + Log_{10} [Volume Per Carrier (mL)]

The percentage of virus inactivation was calculated in the following manner:

[1-Output Viral Load / Initial Viral Load] x 100 = 1-10^ (Log_{10}Reduction Factor) x 100

The Mean Viral Load Log_{10} from n replicates was determined as follows:

$$\text{Mean Viral } Log_{10} \text{ Reduction} = \frac{VL_1 + VL_2 + \ldots\ldots + VL_n}{n}$$

RESULTS (continued)

Table 1
Titer Results

Sample	Replicate	Contact time	Titer ($Log_{10}TCID_{50}/mL$)	Volume (mL)	Viral Load ($Log_{10}TCID_{50}$)
Cell viability/media sterility control	NA		no virus detected, cells viable; media sterile		
Virus Stock Titer Control			7.75	-	-
Theoretical load [a]					7.05
STR-Solution	1	1 hour	5.25	0.2	4.55
	2		5.00	0.2	4.30
	3		4.50	0.2	3.80
	1	2 hours	4.00	0.2	3.30
	2		4.00	0.2	3.30
	3		3.75	0.2	3.05
	1	4 hours	3.00	0.2	2.30
	2		2.30	0.2	1.60
	3		2.75	0.2	2.05
Initial Plate Recovery Control (T = 0 hours)	1	0 hours	7.00	0.2	6.30
	2		7.25	0.2	6.55
	3		7.25	0.2	6.55
	Average				6.48
Final Plate Recovery Control (T = 4 hours)	1	4 hours	5.75	0.2	5.05
	2		6.00	0.2	5.30
	3		6.50	0.2	5.80
	Average				5.50

a The theoretical load is determined based on the Virus Stock Titer control and the volume of virus challenged per carrier.
NA = Not applicable

RESULTS (continued):

Table 2
Cytotoxicity Controls

Dilution of the Neutralized Sample	Cytotoxicity Control
10^{-2}	no cytotoxicity observed in 4 out of 4 wells
10^{-3}	no cytotoxicity observed in 4 out of 4 wells
10^{-4}	no cytotoxicity observed in 4 out of 4 wells

Table 3
Viral Reduction

Test Agent	Contact Time	Replicate Number	Initial Viral Load* ($Log_{10}TCID_{50}$)	Output Viral Load ($Log_{10}TCID_{50}$)	Log_{10} Reduction	Percent Reduction
STR-Solution	1 hour	1	6.48	4.55	1.93	98.829%
		2		4.30	2.18	99.342%
		3		3.80	2.68	99.792%
		Mean Reduction			**2.38**	99.582%
	2 hours	1	6.48	3.30	3.18	99.934%
		2		3.30	3.18	99.934%
		3		3.05	3.43	99.963%
		Mean Reduction			**3.28**	99.948%
	4 hours	1	6.48	2.30	4.18	99.993%
		2		1.60	4.88	99.999%
		3		2.05	4.43	99.996%
		Mean Reduction			**4.59**	99.997%

* Results represent the average of three replicates.

CONCLUSIONS

When tested as described, STR-Solution exhibited a 2.38, 3.28 and 4.59 Log_{10} (99.582%, 99.948%, 99.997%) reduction when Middle East Respiratory Syndrome Coronavirus (MERS-CoV), containing 5% serum, was exposed to the test device for 1 hour, 2 hours and 4 hours respectively at 20°C. All of the controls met the criteria for a valid test. These conclusions are based on observed data.

Study director: _____ Date 6/30/16
Cory Chiossone

APPENDIX

MicroBioTest Protocol

Virucidal Efficacy Test for A Test Device -

Middle East Respiratory Syndrome Coronavirus (MERS-CoV)

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

Prepared for
Forward Medi Co,.Ltd.
HangGang Xi Tower B-dong
Room 804, 1498 Gayang-dong,
Gangseo-Gu, Seoul, Korea

May 3, 2016

MicroBioTest Protocol: FOR.1a.04.28.16

MicroBioTest Project: 922-101

OBJECTIVE:

This test is designed to evaluate the efficacy of a test device against Middle East Respiratory Syndrome Coronavirus (MERS-CoV) using Petri dish glass carriers. The test follows the principles in the "Germicidal Spray Products as Disinfectants" method in the AOAC Official Methods of Analysis (19th Edition, 2013), and the ASTM International test method designated E1053-11, "Standard Test Method to Assess Virucidal Activity of Chemicals Intended for Disinfection of Inanimate, Nonporous Environmental Surfaces" with customization for this test.

TESTING CONDITIONS:

Virus will be dried on Petri dish glass carriers under ambient temperature. Three carriers will be used for device treatment contact time 1, three carriers will be used for device treatment contact time 2, and three carriers will be used for device treatment contact time 3. Additionally, three carriers will be used as the Initial Plate Recovery Control without device treatment or holding; and three carriers will be used as the Final Plate Recovery Control with virus dried and held for the longest contact time without device treatment.

The test carriers will be placed about 5cm from the device. The carriers are positioned vertically with the dried virus films facing the device. The device is powered "on" in accordance with the manufacturer or Sponsor's instruction. After each exposure (contact time), a virus recovery medium (= neutralizer) will be added onto each carrier and the virus particles will be scraped off from the surface and assayed to determine the quantity of remaining infectious virus. Multiple carriers may be treated simultaneously by the same device. The average viral load from three test carriers will be compared to the average of the control carriers to determine the Log10 and percent reduction at each of the contact times.

MATERIALS:

A. Test, control and reference substances, as applicable, will be supplied by the sponsor of the study (see last page). As per CFR 40.160.105:

- The identity, strength, purity, and composition, or other characteristics which will appropriately define the test, control, or reference substance, as applicable, shall be determined for each batch and shall be documented by

the sponsor before its use in a study. Methods of synthesis, fabrication, or derivation of the test, control, or reference substance shall be documented and retained by the sponsor.

- When relevant to the conduct of the study the solubility of each test, control, or reference substance shall be determined by the sponsor before the experimental start date. The stability of the test, control, or reference substance shall be determined by the sponsor before the experimental start date or concomitantly according to written standard operating procedures, which provide for periodic analysis of each batch.

The test substance will be tested as supplied by the sponsor unless directed otherwise. All operations performed on the test substance 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 substance has been appropriately tested for identity, strength, purity, stability, and uniformity as applicable.

MicroBioTest will retain all unused chemical test substances for a period of one year upon completion of the test, and then discard them in a manner that meets the approval of the safety officer, or return them to the Sponsor. The test materials and the paper records will be retained in accordance to FIFRA. MicroBioTest will contact the Study Sponsor to arrange for transfer of records when/if the test substance is returned to the Sponsor.

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

1. Challenge virus (requested by the sponsor of the study): Middle East Respiratory Syndrome Coronavirus (MERS-CoV), BEI Resources

    - Note: the virus inoculum will contain 5% fetal bovine serum.

2. Host cell line: Vero E6, ATCC CRL-1586

3. Laboratory equipment and supplies.

- Clean, sterile 100 x 15 mm plastic Petri dishes
- Disposable sterile cell scrapers
- Sterile serological pipettes
- Micro-pipettors and sterile pipette tips
- 24-well cell culture plates
- Cell incubators
- Autoclave
- Certified clock
- Certified digital timer

4. Media and reagents:

- Cell culture medium (= Virus Recovery Medium)
- Dilution medium
- Sterile deionized water

Details of the media and reagents relevant to the virus-host system and test substance being tested will be documented in the first project sheet and data pack.

B. Materials supplied by the sponsor:

1. Test device

TEST SYSTEM IDENTIFICATION:

All applicable carriers, dilution tube racks, and host-containing apparatus will be appropriately labeled with the following information: virus, host, and test substance and/or project number.

EXPERIMENTAL DESIGN:

The procedures involved in performance of this study are described in a detailed series of SOPs that are maintained at MicroBioTest. SOPs and Logs are referred to in the raw data and are required as part of GLP regulations. The study process is described in the following sections.

A. Inoculum preparation:

The stock virus was purchased from reputable sources and may have been propagated at MicroBioTest in Vero E6 cells. Frozen viral stocks will be thawed on the day of the test. If necessary, the stock virus will be added with fetal bovine serum or diluted with media to achieve a final organic load of 5%.

Note: A 3-5 Log_{10} reduction window is targeted for this study.

B. Carrier preparation:

A total of 15 sterile Petri dish glass carriers will be prepared by adding **0.2 mL virus inoculum** per carrier. The virus inoculum will be spread as much as possible with a cell scraper over an area of approximately 4 in^2 that has been marked on the underside of pre-sterilized glass Petri dishes. All inoculated carriers are incubated under ambient temperature in a biosafety cabinet in sterile plastic Petri dishes until visually dry. The clock start and stop time will be recorded for the drying time of virus. The temperature and humidity will also be recorded.

Nine carriers will be prepared for test device treatment, three for each contact time. Three carriers will be used for the Initial Plate Recovery Control. Three carriers will be used for the Final Plate Recovery Control.

Additionally, one carrier will be prepared for the cytotoxicity control using dilution medium (DM) in lieu of virus as the inoculum. No neutralizer effectiveness/viral interference control is applicable as the test material is not a chemical.

C. Test device preparation and handling:

The test device will be assembled, if required, and operated safely according to the manufacturer or sponsor's instructions as provided.

D. Test:

Note: The temperature and humidity level of the laboratory during the test phase will be monitored and reported.

After the inoculation and drying, the test carriers will be placed about 5 cm underneath the device. The carriers are positioned vertically with the dried virus films facing the device. The device is powered "on" in accordance with the manufacturer or Sponsor's instruction. The carriers will be exposed to the test device throughout the entire exposure (contact time). Note: Multiple carriers may be treated simultaneously by the same device. After each contact time, **2.0 mL** virus recovery medium (= neutralizer) will be added onto each carrier and the virus/neutralizer mixture will be scraped off from the surface of the carrier with a cell scraper. This "post-neutralized sample" (PNS), considered 10^{-1} dilution from the original viral inoculum, will be serially 10-fold diluted in DM. Selected dilutions of the sample will be inoculated onto cultured cell monolayers as described in "Infectivity Assay" section.

E. Controls:

1. Initial Plate recovery control (Initial PRC):

This control will be performed in three replicates concurrently with the test substance runs. The virus inoculum will be spread over the surface of the carrier and left to dry at ambient temperature. Immediately after drying – without device treatment or holding - each carrier will be applied with 2.0 mL of the virus recovery medium and processed as the test. Selected dilutions of the sample will be added to cultured cell monolayers at a minimum of four wells per dilution per sample, as described in the "Infectivity Assay" section. This control will determine the relative loss in virus infectivity resulting from drying and neutralization alone.

The average viral load from the three Initial PRC carriers will be used as the baseline and compared with the test carrier results to determine the Log_{10} and percent reduction by the test device.

2. Final Plate recovery control (Final PRC):

This control will be performed in three replicates concurrently with the test substance runs. The virus inoculum will be spread over the surface of the carrier and left to dry at ambient temperature. The carrier will then be held for the longest contact time as for the test carriers but without any device treatment. Post contact time, the carrier will be applied with 2.0 mL of the virus recovery medium and processed as the test. Selected dilutions of the sample will be added to cultured cell monolayers at a minimum of four wells per dilution per sample, as described in the "Infectivity Assay" section. This control will determine the relative loss in virus infectivity resulting from drying and neutralization alone.

3. Cytotoxicity control:

Although the test material is not chemical, a cytotoxicity control is necessary to confirm that the cytopathic effect (CPE) observed, if any, is from viral infection and not from non-specific cytotoxicity.

One carrier will be used for this control. Dilution medium, in lieu of virus, will be spread over the surface of the carrier and dried. The carrier will be placed next to the three virus-inoculated test carriers onto the cart and subject to the same device treatment for the longest contact time (as a worst-case scenario for the shorter contact times). After the treatment, 2.0 mL of virus recovery medium will be added to the carrier and the residues will be scraped off from the carrier into a collection dish. The sample will be serially 10-fold diluted. Selected dilutions will be added to cultured cell monolayers at four wells per dilution, and incubated along with the other test and control samples. At the end of the incubation, it will be observed for cell condition.

4. Cell viability control:

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. At least four wells of cells will receive

only DM and will be incubated and processed with both test and other controls. This will serve as the negative control.

5. Virus Stock Titer control (VST):

An aliquot of the virus used in the study will be directly serially diluted and inoculated onto the host cells at 4 or 8 replicate wells per dilution 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.

F. Infectivity assay:

The amount of infectious virus in both test and control samples will be assayed by the 50% tissue culture infectious dose assay (TCID50) based on viral-induced cytopathic effect (CPE).

Indicator cells will be seeded into 24-well cell culture plates at an appropriate concentration approximately 12 – 30 hours prior to inoculation.

Following aspiration of the growth media, selected dilutions of the test and control samples will be added to cultured host cells at 1.0 mL per well and 4 wells per dilution.

The inoculated culture will be incubated at 36±2°C with 5±1% CO_2 for total 4 – 9 days. The cells will be observed and refed with fresh media as necessary, during the incubation period. At the end of incubation, the cell culture will be examined microscopically for presence of infectious virus. The resulting virus-specific CPE and test substance-specific cytotoxic effects, if any, will be scored by examining both test and controls. These observations will be recorded.

G. Calculation:

The 50% tissue culture infectious dose per mL ($TCID_{50}$/mL) will be determined using the method of Spearman-Karber. The test results will be reported as the reduction of the virus titer due to treatment with test substance expressed as $\log_{10}$. No statistical analysis will be used for this test.

The Virus Load will be calculated in the following manner:
Virus Load (Log_{10} $TCID_{50}$) = Virus Titer (Log_{10} $TCID_{50}$/mL) + Log_{10} [Volume per sample (mL)]

The Log_{10} Reduction Factor (LRF) will be calculated in the following manner:
Log_{10} Reduction Factor = Initial viral load (Log_{10} $TCID_{50}$) – Output viral load (Log_{10} $TCID_{50}$)

The percentage of virus inactivation was calculated in the following manner:
[1 - Output Viral Load/Initial Viral Load] x 100 = [1 - $10^{(-Log_{10}\ Reduction\ Factor)}$] x 100

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 average viral load recovered from the Initial PRC must be $\geq$ 4.0-log_{10}
- Viral-induced cytopathic effect must be distinguishable from test substance-induced cytotoxic effects (if any).
- Cell viability control and cytotoxicity control must be negative for infectivity.

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, VA 20164.

PROTOCOL AMENDMENTS AND DEVIATIONS:

Any protocol amendment(s) and protocol deviation(s) identified will be reported in project sheet(s) and included in the final report. The sponsor will sign the project sheet(s) to acknowledge the change in the protocol.

STATISTICAL ANALYSIS:

No statistical analysis will be performed in this study.

REPORT FORMAT:

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

- Sponsor identification
- Test device identification
- Type of assay and project number
- Log10 and percent viral reduction
- Test results presented in tabular form and conclusions
- Methods and evaluation criteria, if applicable
- Dates of study initiation and completion (GLP studies only)
- Signed Quality Assurance and Compliance Statements (GLP studies only)
- Certificate of Analysis (GLP studies only; if provided by the Sponsor)

RECORDS TO BE MAINTAINED:

For all GLP studies, the original signed final report will be sent to the Sponsor. A draft report will be provided to Sponsor for review prior to finalization of the report

All raw data, protocol, protocol modifications, test substance records, copy of final report, and correspondence between MicroBioTest and the sponsor will be stored in the archives at MicroBioTest, 105 Carpenter Drive, Sterling, VA 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 substance; challenge virus and host cell line monolayers used and the type of neutralizers 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 initiation date. All project sheets issued will be forwarded to the study sponsor for appropriate action.

SUMMARY OF SAMPLES TO BE ASSAYED:

No.	Treatment	Contact time	Inoculum	Description
1	Test Device	1 hr	Virus	Test device treated, T= 1 hr, Rep. 1
2				Test device treated, T= 1 hr, Rep. 2
3				Test device treated, T= 1 hr, Rep. 3
4		2 hr		Test device treated, T= 2 hr, Rep. 1
5				Test device treated, T= 2 hr, Rep. 2
6				Test device treated, T= 2 hr, Rep. 3
7		4 hr		Test device treated, T= 4 hr, Rep. 1
8				Test device treated, T= 4 hr, Rep. 2
9				Test device treated, T= 4 hr, Rep. 3
10	None	0 hr	Virus	Initial Plate Recovery Control, T = 0 hr, Rep. 1
11				Initial Plate Recovery Control, T = 0 hr, Rep. 2
12				Initial Plate Recovery Control, T = 0 hr, Rep. 3
13		4 hr		Final Plate Recovery Control, T = 4 hr, Rep. 1
14				Final Plate Recovery Control, T = 4 hr, Rep. 2
15				Final Plate Recovery Control, T = 4 hr, Rep. 3
16	Test Device	4 hr		Cytotoxicity Control
17	N/A	N/A	N/A	Cell viability control
18	N/A	N/A	N/A	Virus stock titer control