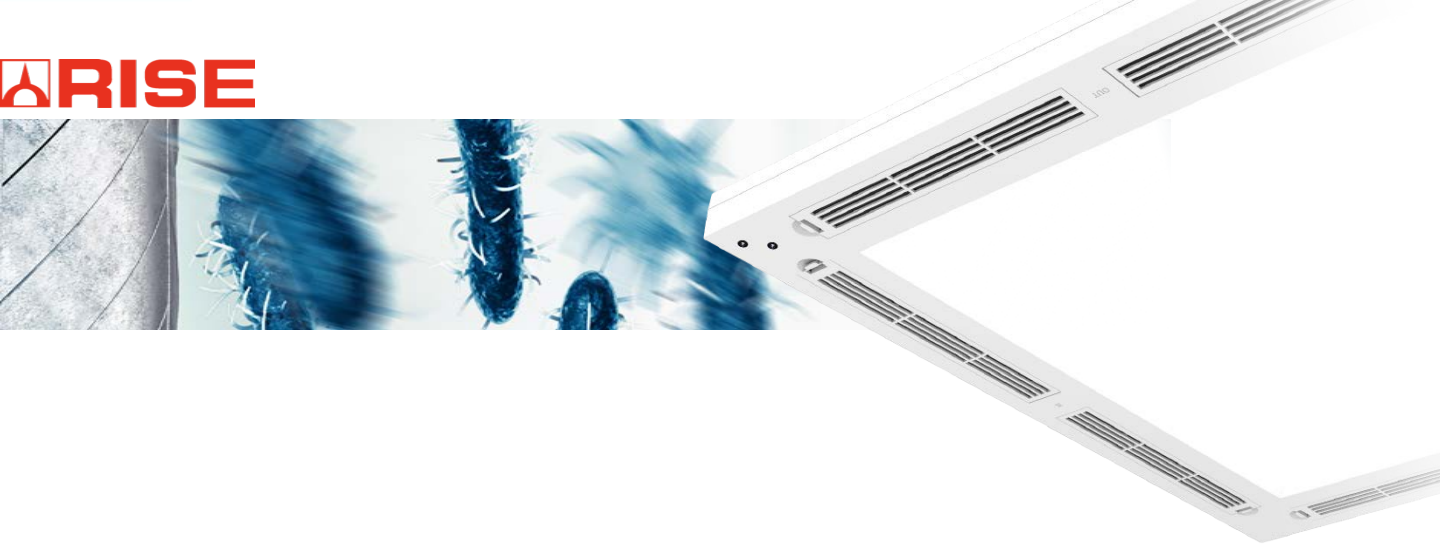

V-Buster

Chapter of TTA (NTDA)

(Nanocomposite Material)

H1N1 測試報告

FINAL REPORT

VIRUCIDAL SUSPENSION EFFICACY TEST
Influenza A Virus (H1N1)

TEST AGENT
Nanocomposite Material

Author
Zheng Chen, M.S.

Performing Laboratory
MicroBioTest
Division of Microbac Laboratories, Inc.

105 Carpenter Drive
Sterling, Virginia 20164

Laboratory Project Identification Number
852-101

Sponsor
JM Material Technology Inc
O. 5F.-3, No. 40-2, Sec. 1, Minsheng N. Rd.
Guishan Township,Taoyuan County 333
Taiwan (R.O.C.)

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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 agent resides with the sponsor of the study.

The following technical personnel participated in this study:

Zheng Chen, Cameron J. Wilde

Study Director: MicroBioTest

Zheng Chen, M.S. — Date: 04/22/2014

QUALITY ASSURANCE UNIT STATEMENT

Title: VIRUCIDAL SUSPENSION EFFICACY TEST – Influenza A Virus (H1N1)

The Quality Assurance Unit of MicroBioTest has inspected the Project Number 852-101 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	04/01/14	04/02/14	04/02/14
In-Process	04/02/14	04/02/14	04/02/14
Final Report	04/10/14	04/10/14	04/10/14

Nathan S. Jones, RQAP-GLP
Quality Assurance Unit — Date: 04/22/14

TEST SUMMARY

TITLE: VIRUCIDAL SUSPENSION EFFICACY TEST – Influenza A Virus (H1N1)

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

TEST MATERIALS:

Nanocomposite Material (JM-TTA01-N000), received at MicroBioTest 02/14/14, assigned DS No. E41

SPONSOR: JM Material Technology Inc
O. 5F.-3, No. 40-2, Sec. 1, Minsheng N. Rd.
Guishan Township,Taoyuan County 333
Taiwan (R.O.C.)

TEST CONDITIONS

Challenge virus:

Influenza A Virus (H1N1), 2009 pandemic H1N1 strain (A/California/04/09), source: Charles River Laboratories

Host:

Madin-Darby canine kidney (MDCK) cells, source: ATCC CCL-34

Active ingredient(s):

TiO_2 & Ag

Neutralizer used:

Eagle's Minimum Essential Medium (MEM) + 1% Fetal Bovine Serum + 0.5% Polysorbate 80 + 1mM EDTA

Dilution medium:

MEM + 3.0 µg/mL Trypsin

Organic load:

5% Serum

UV-A lamp:

365nM, 15W

Contact time(s) Under UV-A lamp at a distance of 35cm:

20 minutes

Test agent application:

Suspension test – direct mixing
(0.3 mL of virus stock added to 2.7 mL of test product)

Contact temperature:

Room temperature (24C actual)

TEST CONDITIONS (continued)

Dilution(s):

Ready to use

Incubation temperature:

36±2C in 5±1% CO_2

Media and reagents:

Eagles Minimum Essential Medium (MEM) + 3.0 µg/mL Trypsin
MEM + 1% Fetal Bovine Serum + 0.5% Polysorbate 80 + 1mM EDTA
Phosphate Buffered Saline
Sephacryl Columns
Fetal Bovine Serum

STUDY DATES AND FACILITIES

The laboratory phase of this test was performed at MicroBioTest, 105 Carpenter Drive, Sterling, VA 20164. Testing was initiated on 04/02/14, and was completed on 04/07/14. The study director signed the protocol on 04/01/14. 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.

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 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

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 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]

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 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

Table 1
Titer Results

Sample	Titer ($Log_{10}TCID_{50}$/mL)	Volume (mL)	Volume Correction[a]	Viral Load ($Log_{10}TCID_{50}$)
Cell viability/media sterility control	no virus detected, cells viable; media sterile			
Virus Stock Titer Control	6.50	-		-
Theoretical viral load per run**	-	-		5.98
Virus Recovery Control (with UV-A)[b]	5.00	3	2	5.78
Virus Recovery Control (without UV-A) [b]	5.75	3	2	6.53
Column Titer Control (with UV-A)	5.25	3	2	6.03
Column Titer Control (without UV-A)	5.75	3	2	6.53
Nanocomposite Materal (with UV-A) [b]	≤ 0.83 *	3	2	≤ 1.61

* No virus was detected. The titer was determined based on the Poisson distribution.
** The theoretical viral load was calculated based on the titer of the stock virus and the volume (0.3 mL) added into each reaction mixture.
[a] Volume correction accounts for the neutralization of the sample post contact time.
[b] Sample was processed by Sephacryl column.

RESULTS (continued)

Table 2
Neutralizer Effectiveness/Viral Interference and Cytotoxicity Controls

Dilution of the Neutralized Sample	Neutralizer Effectiveness/Viral Interference Control (with UV-A) [a]	Cytotoxicity with Control (with UV-A) [a]
10^-1	virus detected in 4 out of 4 wells	no cytotoxicity observed
10^-2	virus detected in 4 out of 4 wells	no cytotoxicity observed
10^-3	virus detected in 4 out of 4 wells	no cytotoxicity observed

[a] Sample was processed by Sephacryl column.

Table 3
Reduction Factor

Test Agent	Contact Time	Initial Viral Load ($Log_{10}TCID_{50}$)	Output Viral Load ($Log_{10}TCID_{50}$)	Log_{10} Reduction	Percent Reduction (%)
Nanocomposite Material	20 minutes	5.78	≤ 1.61	≥ 4.17	≥ 99.99

CONCLUSIONS

MicroBioTest personnel performed the inactivation procedure using Influenza A Virus (H1N1) (A/California/04/09) to spike the test agent solution. Samples were taken and titrated by 50% tissue culture infectious dose ($TCID_{50}$) endpoint assay using MDCK cells.

Table 3 reports the individual Log_{10} virus reduction factor for the test article treatment procedure. All of the controls met the criteria for a valid test. These conclusions were based on observed data.

APPENDIX

Microbac®
LABORATORY SERVICES

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

MicroBioTest Protocol

VIRUCIDAL SUSPENSION EFFICACY TEST

Influenza A Virus (H1N1)

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

Prepared for
JM Material Technology Inc
O. 5F.-3, No.40-2, Sec. 1, Minsheng N. Rd.
Guishan Township, Taoyuan County 333
Taiwan (R.O.C.)

February 12, 2014

MicroBioTest Protocol: JMM.1.02.12.14

MicroBioTest Project: 852-101

OBJECTIVE:

This study is designed to measure virucidal effectiveness of a test agent. If determines the potential of the test agent to inactivate virus in suspension. The test follows the principle outlined in the American Society for Test Materials (ASTM) test method designated E1052 – 11, "Standard Test Method to Assess the Activity of Microbicides against Viruses in Suspension".

TESTING CONDITIONS:

The test agents will be evaluated against the challenge virus in suspension. One test agent, one lot, will be evaluated for inactivation of Influenza A Virus (H1N1) at one exposure (contact) time. Singlet runs (N=1) will be performed for the condition. To minimize buffer interference and to minimize reduction of virucidal activity, the volume of virus inoculum added to test material will be kept to equal or less than 10% of the total volume of the test. Aliquots will be removed at the completion of the contact time from the test agent/virus reaction mixture; neutralized (quenched); and inoculated onto appropriate host cell system. The inoculated host system will be incubated and read for presence of infectious virus.

MATERIALS:

A. Test, control and reference substances will be supplied by the sponsor of the study (see last page).

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

MicroBioTest will retain all unused test agent for a period of at least three months after completion of the test, then return them to the sponsor of the study or 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 of the study): Influenza A Virus (H1N1)

2. Host cell line: MDCK cells

3. Laboratory equipment and supplies.

4. Media and reagents:

Media and reagents appropriate to the virus-host system will be used and documented in the data pack and project sheets.

TEST SYSTEM IDENTIFICATION:

All dilution tube racks and host-containing apparatus will be labeled with the following information: virus and project number.

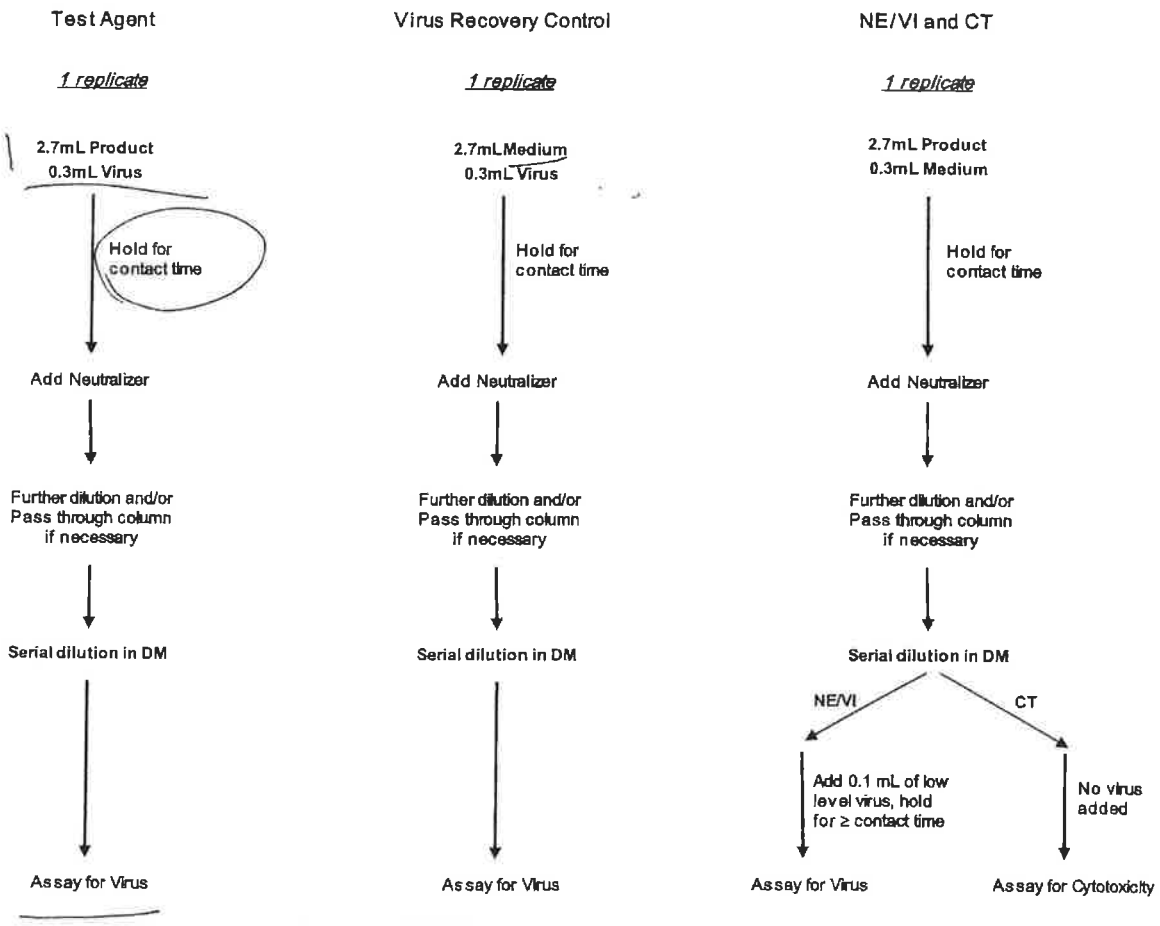
EXPERIMENTAL DESIGN:

All of the procedures involved in the 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 flow diagram is summarized in Figure 1, with details described below.

FIGURE 1

Title: VIRUCIDAL SUSPENSION EFFICACY TEST – Influenza A Virus (H1N1)



NE/VI: Neutralizer Effectiveness/Cytotoxicity Control
CT: Cytotoxicity Control
DM: Dilution Medium

Note: One test agent, one lot, will be evaluated at one contact time. One replicate run (N=1) will be performed for the condition.

A. Inoculum preparation:

Viral stocks are purchased from reputable sources and may have been propagated at MicroBioTest. They are titered and stored in an ultra-low temperature freezer. Records are maintained that demonstrate the origin of the virus.

Frozen viral stocks will be thawed on the day of the test (fresh stock cultures may be used at the discretion of the Study Director).

B. Test agent preparation:

The test agent will be prepared according to the sponsor's directions or label claims (if available).

C. Test:

One test agent, one lot, will be evaluated at one contact time. Singlet runs (N=1) will be performed for the condition.

For the run, a 2.7-mL aliquot of the test agent will be spiked with 0.3 mL of the virus suspension and mixed thoroughly by vortexing. At the completion of the contact time, the entire reaction mixture or an aliquot thereof will be immediately mixed with an equal volume of neutralizer. The post-neutralized sample (PNS) may be further quenched by dilution with dilution medium to remove cytotoxicity. The quenched sample will be serially tenfold diluted with dilution medium (DM) and selected dilutions will be inoculated onto host cells to assay for infectious virus.

If columns are used to further neutralize the test agent and to reduce the cytotoxicity, an aliquot of the PNS sample will be loaded into separate pre-spun Sephacryl columns. The eluates will be aseptically collected and serially diluted in ten-fold increments. If columns are not used, serial ten fold dilutions of neutralized virus-test agent mixture will be prepared in appropriate diluent.

D. Infectivity assay:

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

Selected dilutions of the neutralized inoculum/test agent mixture will be added to cultured cell monolayers at a minimum of four wells per dilution per sample. The host cells may be washed twice with phosphate buffered saline (PBS) prior to inoculation. The inoculated plates will be incubated at 36±2C in 5±1% CO_2 for 4-6 days. The host cell cultures will be observed and refed, as necessary, during the incubation period. These activities, if applicable, will be recorded. Then the host cells will be examined for presence of infectious virus. The resulting virus-specific CPE and test agent-specific cytotoxic effects will be scored by examining both test and controls. These observations will be recorded.

E. Controls:

1. Neutralizer effectiveness/viral interference control:

This control will determine if residual active ingredient is present after neutralization and if the neutralized test agent interferes with virus infectivity. This control will be performed for the test agent in singlet runs.

A 2.7-mL aliquot of the test agent will be mixed thoroughly with 0.3 mL of medium by vortexing, held for contact time, and then neutralized by adding an equal volume of neutralizer. The neutralized mixture will be serially tenfold diluted using dilution medium. The dilution will be divided into two portions, one for Neutralizer effectiveness/viral interference control, and the other for cytotoxicity control

For the Neutralizer effectiveness/viral interference control, 100 µL of a low titered virus stock, containing no more than approximately 5,000 infective units, will be added to 4.5 mL of each of the selected dilutions of the sample and held for a period equivalent or greater than the longer contact time. The virus-spiked solution will be used to inoculate host cells as described for the test procedure.

2. Cytotoxicity control:

   This control will be performed for the test agent in singlet runs.

   Selected dilutions of the sample obtained from the Neutralizer effectiveness/viral interference control run will be inoculated onto host cells and incubated together with other test and control samples as described for the test procedure. The condition of the host cells will be recorded at the end of the incubation period. The cytotoxic effects should be distinct from virus-specific cytopathic effects, which will be evident in the stock titer and virus recovery control cultures.

3. Virus recovery control (VRC):

   This control will be performed in singlet. A 2.7-mL aliquot of the medium will be spiked with 0.3 mL of virus suspension and thoroughly mixed by vortexing. After the contact time, an aliquot of the reaction mixture will be mixed with an equal volume of neutralizer. The neutralized sample will be serially tenfold diluted with dilution medium and selected dilutions will be inoculated onto host cells to assay for infectious virus. The virus control results from this control will be used as the input viral load and compared with the test agent treatment results to evaluate viral reduction by the test agent.

4. Column titer control (to be performed only if a Sephacryl column is used):

   This control will be performed to determine any affect the columns may have on infectious virus titer.

   The sample for this control will be acquired from a portion of the VRC, prior to passing through the columns and will be serially diluted in DM, then processed in the same manner as the test.

5. Cell viability control:

   At least four wells will be inoculated with an appropriate media during the incubation phase of the study. This control will demonstrate that cells

remain viable throughout the course of the assay period. In addition, it will confirm the sterility of the media employed throughout the assay period.

6. 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 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. Calculation:

The 50% tissue culture infective dose per mL ($TCID_{50}$/mL) will be determined using the method of Spearman-Karber (Kärber G. Arch. Exp. Pathol. Pharmakol. Vol. 162. Pages: 480-483, 1931) or other appropriate methods such as Reed and Muench, Am. J. of Hyg. 1938, 27:493. 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 agent 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.

- Virus must be recovered from the neutralizer effectiveness/viral interference control (not exhibiting cytotoxicity).
- Viral-induced CPE must be distinguishable from test agent induced toxicity.
- Cell Viability Control must not exhibit viral-induced CPE or cytotoxicity.

PERSONNEL AND TESTING FACILITIES:

A study director will be assigned before initiation of the test. Resumes for technical personnel are maintained and are available on request. This study will be conducted at MicroBioTest, 105 Carpenter Drive, Sterling VA 20164.

REPORT FORMAT:

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

- Sponsor identification and test material identification
- Type of test 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)

RECORDS TO BE MAINTAINED:

All raw data, protocol, protocol modifications, test agent 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 agent; 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 the 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.

MISCELLANEOUS INFORMATION:

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

A. Name and address: JM Material Technology Inc
O. 5F.-3, No.40-2, Sec. 1, Minsheng N. Rd.
Guishan Township, Taoyuan County 333
Taiwan (R.O.C.)

B. Test agent: Nanocomposite Material

Active ingredient(s): TiO_2 & Ag

Lot No.: NA.

Exposure (Contact) time: ☑ 20 min

Exposure temperature: ☑ Room temperature or ☐ _____ ±2C

Dilution to be tested: ☑ Ready to use or ☐ _____

Diluent: ☑ not applicable
☐ sterile deionized water
☐ Other _____

C. Precautions/storage conditions: refer to MSDS or certificate of analysis
☑ provided ☐ not provided

D. Organic Load: ☐ N/A or ☑ 0 % Serum

REPORT HANDLING:

The sponsor intends to submit this information to:

☐ EPA ☑ FDA ☐ other _____

STUDY CONDUCT: ☑ GLP

Continue on next page

MISCELLANEOUS INFORMATION (continued):

PROTOCOL APPROVAL BY SPONSOR

Sponsor Signature: Chung Chen, Lan Date: 2014/2/26

Printed Name: CHUNG-CHEN, LAN

PROTOCOL APPROVAL BY STUDY DIRECTOR (MicroBioTest)

Study Director Signature: _____ Date: 04/01/14

Printed Name: Zheng Chen

Date Issued: 04/01/14 Project Sheet No. 1 Page No. 1 Laboratory Project Identification No. 852-101

STUDY TITLE: VIRUCIDAL SUSPENSION EFFICACY TEST – Influenza A Virus (H1N1)	**STUDY DIRECTOR:** Zheng Chen, M.S. Signature 04/02/2014 Date		
TEST MATERIAL(S): Nanocomposite Materal (JM-TTA01-N000)	**LOT NO.** NA	**DATE RECEIVED:** 02/14/14	**DS NO.** E41
PERFORMING DEPARTMENT(S): Virology and Molecular Biology	**STORAGE CONDITIONS:** Location: J1 ■ Dark ■ Ambient Room Temperature ☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:		
PROTECTIVE PRECAUTION REQUIRED: MSDS ☐Yes / ■ No			
PHYSICAL DESCRIPTION: ☐ Solid ■ Liquid ☐ Aerosol ☐ Other:			
PURPOSE: See attached protocol. **AUTHORIZATION:** See client signature.			
PROPOSED EXPERIMENTAL START DATE: 04/02/14 **TERMINATION DATE:** 04/09/14			
CONDUCT OF STUDY: ■ FDA ☐ EPA ☐ R&D ■ GLP ☐ GCP ☐ Other:			
SPONSOR: JM Material Technology Inc O.5F.-3, No.40-2,Sec.1, Minsheng N. Rd. Guishan Township,Taoyuan County 333 Taiwan(R.O.C.)	**CONTACT PERSON: Chung Chen, Lan** E-mail: shain0204@hotmail.com		

TEST CONDITIONS:

Challenge organism: Influenza A Virus (H1N1), 2009 pandemic H1N1 strain (A/California/04/09), Charles River Laboratories

Host cell line: Madin-Darby canine kidney (MDCK) cells, ATCC CCL-34

Active ingredient(s): TiO_2 & Ag

Organic load: 5% Serum

Dilution medium: Eagle's Minimum Essential Medium (MEM) + 3.0 µg/mL Trypsin

Neutralizer: MEM + 1% Fetal Bovine Serum (FBS) +0.5% Polysorbate 80 + 1mM EDTA

Contact time(s): 20 minutes

Contact temperature: Ambient Room Temperature

Test Agent Concentration Tested: Ready-to-use

Incubation Temperature(s): 36±2°C and 5±1%CO_2 **Incubation time:** 4 – 6 days

Continued on page 2

EXPLANATION:

This project sheet was issued to document the following:

Protocol Amendment(s):

1. Page 10; section D (Organic Load) of protocol states: "0% serum". Per client request, the 5 % serum will be used in the test. This amendment serves to clarify the protocol.

2. Per client request the test will be performed as "after mixing, the virus/test agent reaction mixture will be irrdiated under a UV-A lamp which has a wavelength of 365nm and a power of 15w for 20 min (i.e., throughout the entire contact time), at a distance of 35cm. After the irradiation, the reaction mixture will immediately be neutralied and then assayed. The Virus Recovery Control will undergo the same irradiation procedure during the contact time before neutralization. This amendment serves to clarify the page 4, page 5 and page 7 of the protocol.

3. An additional control, "Virus Recovery Control without UV" will be performed, wherein the virus/medium mixture will NOT be UV irradiated during the contact time. This amendment serves to add an additional control to the protocol.

4. Protocol, Page 10 (Section C), states "MSDS or certificate of analysis" provided, however, the sponsor did not provide MSDS or certificate of analysis. This amendment serves to clarify the protocol.

Date Issued: 04/22/14 Project Sheet No. 2 Page No. 1 Laboratory Project Identification No. 852-101

STUDY TITLE: VIRUCIDAL SUSPENSION EFFICACY TEST – Influenza A Virus (H1N1)	**STUDY DIRECTOR:** Zheng Chen, M.S. Signature 04/22/14 Date		
TEST MATERIAL(S): Nanocomposite Material (JM-TTA01-N000)	**LOT NO.** NA	**DATE RECEIVED:** 02/14/14	**DS NO.** E41
PERFORMING DEPARTMENT(S): Virology and Molecular Biology	**STORAGE CONDITIONS:** Location: J1 ■ Dark ■ Ambient Room Temperature ☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:		
CONDUCT OF STUDY: ■ FDA ☐ EPA ☐ R&D ■ GLP ☐ GCP ☐ Other:			
SPONSOR: JM Material Technology Inc O.5F.-3, No.40-2,Sec.1, Minsheng N. Rd. Guishan Township,Taoyuan County 333 Taiwan(R.O.C.)	**CONTACT PERSON: Chung Chen, Lan** E-mail: shain0204@hotmail.com		

EXPLANATION:

This project sheet was issued to document the following:

Protocol Amendment(s):

5. On Project Sheet no. 1 under Test Material(s) was stated "Nanocomposite Materal". This is a typographical error. The correct test material was "Nanocomposite Material". This amendment serves to correct the typographical error on Project Sheet no. 1.