FINAL REPORT

VIRUCIDAL SUSPENSION EFFICACY TEST
Enterovirus

TEST AGENT
Nanocomposite Material

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

Laboratory Project Identification Number
852-102

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, Semhar Fanuel

Study Director: MicroBioTest (1) 04/30/2014

Zheng Chen, M.S. Date

QUALITY ASSURANCE UNIT STATEMENT

Title: VIRUCIDAL SUSPENSION EFFICACY TEST – Enterovirus

The Quality Assurance Unit of MicroBioTest has inspected the Project Number 852-102 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	04/02/14	04/02/14	04/02/14
In-Process	04/02/14	04/02/14	04/02/14
Final Report	04/25/14	04/25/14	04/25/14

Nathan S. Jones 04/30/14

Nathan S. Jones, RQAP-GLP Date
Quality Assurance Unit

(1) Signed for Z.C. SF 04/30/2014

TEST SUMMARY

TITLE: VIRUCIDAL SUSPENSION EFFICACY TEST – Enterovirus

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.
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TEST CONDITIONS

Challenge virus:

Human Enterovirus Type 71, ATCC VR-1432

Host:

LLC-MK2 cells, ATCC CCL-7.1

Active ingredient(s):

TiO_2 & Ag

Neutralizer used:

RPMI 1640 + 1% Newborn Calf Serum (NCS) + 0.5% Polysorbate 80 + 1mM EDTA

Dilution medium:

RPMI 1640 + 2% NCS

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 (24°C actual)

TEST CONDITIONS (continued)

Dilution(s):

Ready to use

Incubation temperature:

36±2C in 5±1% CO_2

Media and reagents:

RPMI 1640 + 2% Newborn Calf Serum (NCS)
RPMI 1640 + 1% NCS + 0.5% Polysorbate 80 + 1mM EDTA
Sephacryl Columns

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 04/16/14. 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 Factor]

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	5.75	-		-
Theoretical viral load per run**	-	-		5.23
Virus Recovery Control (with UV-A)[b]	5.00	3	2	5.78
Virus Recovery Control (without UV-A)[b]	4.75	3	2	5.53
Column Titer Control (with UV-A)	5.25	3	2	6.03
Column Titer Control (without UV-A)	5.00	3	2	5.78
Nanocomposite Material (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 Enterovirus to spike the test agent solution. Samples were taken and titrated by 50% tissue culture infectious dose ($TCID_{50}$) endpoint assay using LLC-MK2 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