

# ANTIMICROBIAL TEST LABORATORIES

## Study Report NG4331: Modified JIS Z 2801 Test for Antimicrobial Activity and Efficacy

### Against Human Coronavirus 229E

Page 1 of 5

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#### Client Information

Company Name:	<u>NanoTouch Materials, LLC</u>	Sponsor:	<u>Dennis Hackemeyer</u>
Sponsor's Phone:	<u>(888) 411-6843, Ext. 101</u>	E-mail:	<u>dennis@nanotouchmaterials.com</u>

#### Test Information

Test(s) Performed:	<u>Modified JIS Z 2801 Test for Antimicrobial Activity and Efficacy (Study ID NG4331)</u>
Performed by:	<u>Luisa Ikner, Ph.D.</u>

#### Sample Information

Sample Receipt Date(s):	<u>27 June 2013</u>	Internal Code:	<u>2082</u>
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#### Parameters

Virus, Strain:	<u>Human coronavirus, 229E</u>	Exposure Temp.	<u>Ambient (24 to 25 °C)</u>
Host Cell Line:	<u>MRC-5, ATCC CCL-171</u>	Type of Carrier:	<u>1" x 1" NanoSeptic IV</u>
Host Cell Passage #:	<u>34 (From Initial Deposit)</u>	Test/Assay Medium:	<u>See Notes Below.</u>
Lab Virus Stock ID:	<u>HCoV14MAR2012</u>	# of Replicates:	<u>Duplicate</u>
Contact Time(s):	<u>(4): 30 Min, 1 Hour, 2 Hours, 4 Hours</u>	Incubation Temp.:	<u>35.0 ± 1°C</u>
Soil Load:	<u>0% Soil Load</u>	Incubation Time:	<u>7 Days</u>
Neutralizer Used:	<u>2% FBS EMEM plus Antibiotics</u>		

#### Controls

Ctrl Titer Avg / Carrier:	<u>T=0: 5.43 log<sub>10</sub>; T=30 Min: 5.18 log<sub>10</sub>; T=1 Hr: 5.18 log<sub>10</sub>; T=2 Hrs: 4.80 log<sub>10</sub> and T=4 Hrs: 4.68 log<sub>10</sub></u>
Cytotoxicity Control:	<u>No cytotoxicity observed on MRC-5's for Stainless Steel Ctrl and NanoSeptic IV surfaces.</u>
Neutralization Control:	<u>Viral CPE observed in all dilutions 10<sup>-1</sup> to 10<sup>-3</sup> for Stainless Steel Control and NanoSeptic IV surfaces.</u>
Sterility Control:	<u>No contamination observed.</u>

**Notes:** The Test/Assay medium used during the study was EMEM supplemented with 2% FBS plus antibiotics.

The cell culture virucidal efficacy assay was based on EPA-approved methodology. Viral titers were determined using the Spearman-Karber Method.

Test Completed:	<u>10-July-2013</u>	Report Sent:	<u>16-July-2013</u>
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# ANTIMICROBIAL TEST LABORATORIES

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### Against Human Coronavirus 229E

Page 2 of 5

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### Human Coronavirus Results

**Table 1. Modified JIS Z 2801 Test Data: Evaluation of NanoSeptic IV Against Human Coronavirus 229E<sup>a, b</sup>**

Microorganism	Carrier Type	Contact Time	Log <sub>10</sub> IU/Carrier	Mean Log <sub>10</sub> IU/Carrier	% Reduction vs Control	% Reduction vs Time Zero
Human coronavirus 229E, ATCC VR-740	Stainless Steel (Control)	Time Zero	5.05	5.43	N/A	N/A
			5.80			
	Stainless Steel (Control)	30 Minutes	4.80	5.18	N/A	43.8%
			5.55			
	NanoSeptic IV (Test)		≤ 1.80	≤ 1.80	≥ 99.96%	≥ 99.98%
			≤ 1.80			
	Stainless Steel (Control)	1 Hour	5.05	5.18	N/A	76.6%
			5.30			
	NanoSeptic IV (Test)		≤ 1.80	≤ 1.80	≥ 99.96%	≥ 99.98%
			≤ 1.80			
	Stainless Steel (Control)	2 Hours	4.80	4.80	N/A	76.6%
			4.80			
	NanoSeptic IV (Test)		≤ 1.80	≤ 1.80	≥ 99.90%	≥ 99.98%
			≤ 1.80			
	Stainless Steel (Control)	4 Hours	4.80	4.68	N/A	82.2%
			4.55			
NanoSeptic IV (Test)	≤ 1.80		≤ 1.80	≥ 99.87%	≥ 99.98%	
	≤ 1.80					

<sup>a</sup>"IU" = Infectious Viral Units

<sup>b</sup>"≤" = No human coronavirus detected; viral levels at or below limit of detection.

# ANTIMICROBIAL TEST LABORATORIES

Study Report NG4331: Modified JIS Z 2801 Test for Antimicrobial Activity and Efficacy

Against Human Coronavirus 229E

Page 3 of 5

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Human Coronavirus Results, Continued.

**Table 2. Log<sub>10</sub> Reduction Values**

Contact Time	Log <sub>10</sub> Reduction vs. Control	Log <sub>10</sub> Reduction vs. Time Zero
30 Minutes	≥ 3.38	≥ 3.63
1 Hour	≥ 3.38	≥ 3.63
2 Hours	≥ 3.00	≥ 3.63
4 Hours	≥ 2.88	≥ 3.63

**Table 3. Environmental Parameters**

Contact Time	Time Recorded	Room Temperature (°C)	Relative Humidity	Illuminance (lux)
30 Minutes	1146	24.3	36%	1476
1 Hour	1217	24.6	36%	1472
2 Hours	1319	24.7	36%	1482
4 Hours	1518	24.5	36%	1464

# ANTIMICROBIAL TEST LABORATORIES

## Study Report NG4331: Modified JIS Z 2801 Test for Antimicrobial Activity and Efficacy

### Against Human Coronavirus 229E

Page 4 of 5

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### **Summarized Test Procedure**

#### Preparation and Inoculation of Carriers

- The Study Sponsor-provided sheets of NanoSeptic IV measuring ~8.5" x 11"; the sheets were aseptically cut to squares measuring ~ 1" x 1".
- For T=0 and each of the contact times requested (T=30 Min, 1 Hour, 2 Hours, and 4 Hours), stainless steel control squares (~ 1" x 1") were ethanol-sanitized and double-rinsed in RO water, and then autoclaved prior to testing.
- The carriers were loaded into sterile Petri dishes using sterile forceps.
- A stock vial of human coronavirus 229E was removed from cryostorage the morning of the study to thaw, and 0.010 ml aliquots were aseptically spread over the surface of each test and control carrier to ~1/8 inch of the edge. Virus films were prepared in duplicate per test and control surface, per contact time.
- Control and test carriers were dried with Petri dish lids slightly ajar. Drying times and conditions: 20 minutes, 24.7 °C, 36% Relative Humidity, Illuminance = 1140 lux

#### Test Execution

- The study contact times of 30 Min, 1 Hour, 2 Hours, and 4 Hours were initiated when the test and control carriers were visibly dry. The Petri dish lids were removed for the duration of the study contact time(s).
- At the close of the each study contact time, the test and control carriers were aseptically transferred to tubes containing 2.0 ml of neutralizing solution (2% FBS EMEM).
- The carriers were vortexed for 30 seconds each to mechanically dislodge the microorganisms for enumeration. The inoculated sides of each carrier were further treated using a cell scraper to ensure adequate removal of the test viruses.
- For cytotoxicity and neutralization effectiveness controls, one test and one control carrier each (with no virus film) were each aseptically transferred to neutralization tubes, and vortexed as described previously for the virus test and control films. Approximately 3-log<sub>10</sub> of virus (low titer) was added to each neutralization aliquot prior to plating.
- The vortexed suspensions were serially diluted ten-fold in neutralizing solution, and selected dilutions were plated in quadruplicate onto the appropriate host cell monolayers prepared to suitable confluency in multi-well trays.
- Virus control, cytotoxicity, neutralization validation, and sterility controls were performed concurrently
- Virus reductions were calculated using the Spearman-Kärber Method, and reported to the Study Sponsor.

### **Study References**

JIS Z 2801:2000. Antimicrobial Products - Test for Antimicrobial Activity and Efficacy. Japanese Standards Association. Tokyo, Japan.

# ANTIMICROBIAL TEST LABORATORIES

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Against Human Coronavirus 229E

Page 5 of 5

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## Study Photographs

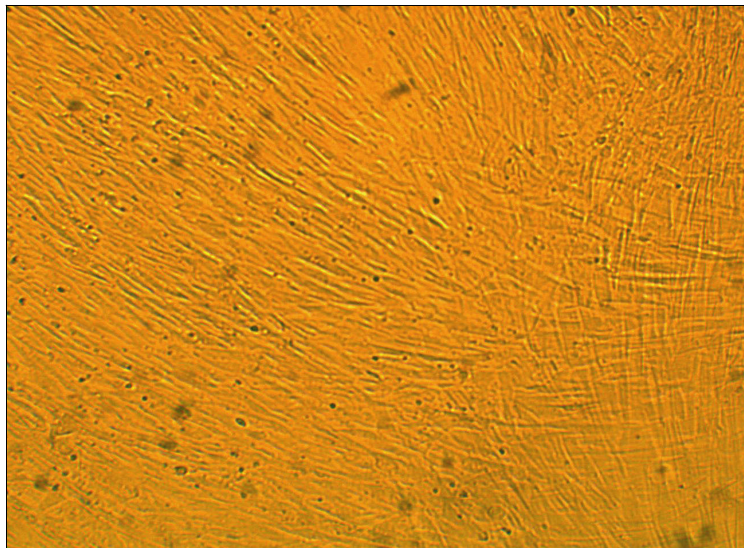


Photo 1. Healthy MRC-5 host cell monolayer.

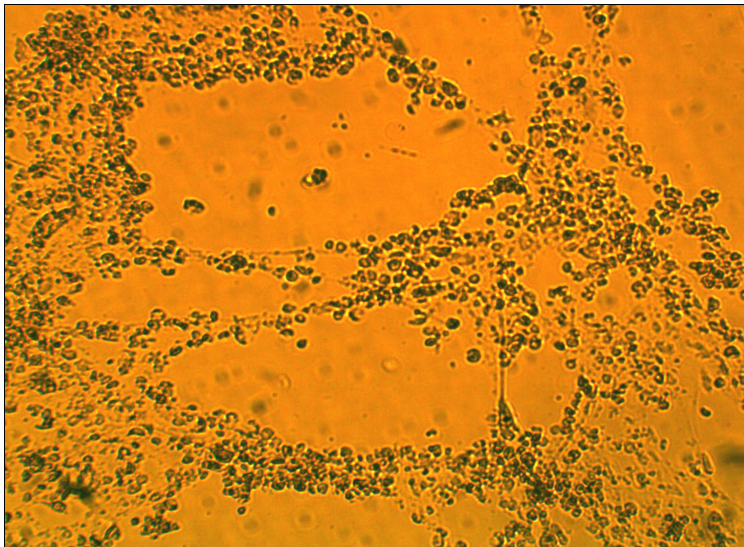


Photo 2. Advanced viral cytopathic effects (CPE) due to infection of MRC-5 host cells by human coronavirus 229E.