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Cathay General Hospital

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Test Report

Efficacy of A New JM Nanocomposite Material in Inhibiting Respiratory Syncytial Virus Cellular Infection

Test Reagent
New JM Nanocomposite Material

Project Commissioner
JM Material Technology, Inc.

Project Implementation Unit
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Signature: 

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Abstract

Title: Efficacy of A New JM Nanocomposite Material in Inhibiting Respiratory Syncytial Virus Cellular Infection

Experiment design: This project conducted laboratory tests on the efficacy of a JM nanomaterial in inhibiting the cellular infection of the respiratory syncytial virus in a virus suspension. A $TCID_{50}$ assay was used in an antivirus test to observe the cytopathic effect of infected cells in JM nanomaterials treated with a virus-enriched culture fluid to calculate the efficacy of JM nanomaterials inhibiting virus.

Test reagent: New JM nanocomposite material

Reagent vendor: JM Material Technology, Inc., 5F-3, No. 40-2, Sec. 1, Minsheng N Rd., Guishan Township, Taoyuan County

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Test Content

Experiment Materials

Virus strain source:

Respiratory syncytial virus (RSV) sourced from a College of American Pathologists proficiency-testing specimen

Host cell:

BCRC 60013 Vero cell line procured from the Bioresource Collection and Research Center, Taiwan, R.O.C.

Experimental Methods

1. Cell culture
   (a) Inoculate the Vero cell strain in a 24-well culture plate.
   (b) Incubate cells in minimum essential medium (MEM) supplemented with 8% fetal bovine serum at 36 °C with 5% CO_2 for 48 h until fully grown.
   (c) Discard the culture fluid, rinse twice with phosphate buffer saline (PBS), and set aside.
2. Virus preparation
   (a) Inoculate the virus in culture tubes containing cell strains.
   (b) Incubate cells in MEM at 36 °C with 5% CO_2 for 48 h until cytopathy occurs.
   (c) Scrape off the cells and precipitate cells by centrifugation at 6000 rpm for 2 min.
   (d) The supernatant is collected as the virus suspension.
   (e) Add 900 uL of MEM to 100 uL of the virus suspension and dilute it at a ratio of 1:10.
   (f) Add 100 uL of the above dilution to 900 uL of MEM and perform a tenfold serial dilution.

Number	1	2	3	4	5	
MEM（+ Trypsin）	900	900	900	900	900	
suspension	100	0	0	0	0	
Virus suspension		100	100	100	100	100
Final volume	900	900	900	900	900	
Final concentration	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	

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3. $TCID_{50}$ Assay

Control group

Use a 24-well plate with seeded cells. Leave the 4 culture tubes in Column 1 untreated as the control, and treat Column 2 to 6 with the enterovirus by adding 200 uL of virus suspension at 10-, 10^2-, 10^3-, 10^4-, and 10^5-fold dilutions, respectively.

Experimental group

(a) Prepare a 5-fold dilution by adding 100 uL of the virus suspension to 400 uL of MEM.

(b) Prepare a 10-fold serial dilution by adding 50 uL of the dilution to 450 uL of MEM.

(c) Prepare 1.25% disinfectant (75 uL of disinfectant + 5925 uL of MEM) and add 450 uL of the disinfectant to each of the above dilution.

(d) Prepare 450 uL of the 1.25% disinfectant, adding it to 450 uL of virus-free MEM for the JM toxicity test.

Number	BC	B1	B2	B3	B4	B5
MEM（+ Trypsin）	450	400	450	450	450	450
Virus suspension	0	100	0	0	0	0
Serial dilution		50	50	50	50	50	Discarding 50
1.25% Disinfectant	450	450	450	450	450	450
Final Volume	900	900	900	900	900	900
Final concentration of virus	0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Final concentration of disinfectant	0.625%	0.625%	0.625%	0.625%	0.625%	0.625%

(e) Expose the dilution to UV for 1 h at room temperature.

(f) Among the cell strains in the 24-well culture plate, inoculate the four wells in Column 1 for the JM toxicity test; inoculate the remaining five columns with 200 uL of 10^1-, 10^2-, 10^3-, 10^4-, and 10^5-fold diluted virus suspension that is treated with 0.625% of the JM nanomaterial.

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Allow both the experimental group and control group to be infected for 1 h at 36 °C and 5% CO_2, and shake them every 20 min. Add to each culture tube, incubate at 36 °C with 5% CO_2, observe daily for the number of tubes displaying cell pathology. Add 1 mL of 4% formaldehyde and leave them to stand at room temperature for 1 h. Rinse them twice with tap water, add 1 mL of 0.5% crystal violet, and leave them to stand at room temperature for 5 min.

4. Interpretation and Calculation
   (a) The Reed–Muench method was used to calculate $TCID_{50}$.
   (b) Formula for calculating viral inhibitory efficacy:
   Inhibition percentage = [1 - 10^ (- (viral load of the control group (Log10TCID50) - viral load of the experimental group (Log10TCID50)] × 100

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Test Results

Respiratory Syncytial Virus

Group	Viral load ($Log_{10}TCID_{50}$)		
	1st	2nd	3rd
Virus strains	3.0	4.5	4.7
Virus strains + JM	2.5	4.0	3.7
Cell strains	None	None	None
Cell strains + JM	None	None	None

Calculation of viral inhibitory efficacy:
Substituting the values of the third test into the formula obtained the following results:
Respiratory syncytial virus inhibition percentage
= [1 - 10^ (- (4.7 - 3.7)] × 100 = **90.00**

Conclusion

The experiment results show that a 0.625% concentration of the JM nanomaterials inhibit cellular infection of respiratory syncytial viruses. The percentage of viral inhibition was **90.00%**.