國泰綜合醫院
Cathay General Hospital

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Test Report

Efficacy of A New JM Nanocomposite Material in Inhibiting Influenza A (H1N1) Virus Infection

Test Reagent

New JM nanocomposite material

Project Commissioner

JM Material Technology Inc.

Project Implementation Unit

Cell Biology Laboratory, Cathay Medical Research Institute, Department of Medical Research, Cathay General Hospital

Testing Laboratory

Virology Laboratory, Cathay General Hospital, Sijhih Branch

Project Personnel

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Signature: 

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Abstract

Title: Efficacy of A New JM Nanocomposite Material in Inhibiting Influenza A (H1N1) Virus Infection

Experiment design: This study tested the efficacy of a new JM nanocomposite material in inhibiting influenza A virus (H1N1) infection. 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:

Influenza A virus - New Caledonia/20/99 (H1N1) sourced from a College of American Pathologists proficiency-testing specimen

Host cells

MDCK cell strains (BCRC 60004) procured from the Bioresource Collection and Research Center, Taiwan, R.O.C.

Experimental Methods

1. Cell culture
   (a) Inoculate the cell strains in a 24-well culture plate.
   (b) Incubate cells 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 and 2 μg/mL trypsin at 36 °C with 5% CO2 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 1080 uL of MEM to 120 uL of the virus suspension and dilute it at a ratio of 1:10.
   (f) Add 120 uL of the above dilution to 1080 uL of MEM and perform a 10-fold serial dilution.

Number	1	2	…	6	7	8	9
MEM (+trypsin)	900	900	…	900	900	900	900
Virus suspension	100	0	…	0	0	0	0
Serial dilution	 100 100 100 100 100 100						
Final volume	900	900	…	900	900	900	1000
Final concentration	10^{-1}	10^{-2}	…	10^{-6}	10^{-7}	10^{-8}	10^{-9}

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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 10^1-, 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.

(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.

No.	BC	B1	B2	B3	B4	B5
MEM (+trypsin)	450	450	450	450	450	450
Virus suspension	0	100	0	0	0	0
Serial dilution		50	50	50	50	50 Discarding
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%

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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 MEM（+Trypsin）to each culture tube, incubate at 36 °C with 5% CO_2, and 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

1. The Reed–Muench method was used to calculate $TCID_{50}$.
2. Formula for calculating viral inhibitory efficacy:
   percentage of inhibition = [1 - 10^ (-(viral load of the control group (Log10TCID50) - viral load of the experimental group (Log10TCID50)] x 100

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Test results

Influenza A virus (H1N1)

Group	Viral load ($Log_{10}TCID_{50}$)		
	1st	2nd	3rd
Virus strains	4.0	5.7	5.7
Virus strains +JM	2.5	3.2	4.0
Cell strains	None	None	None
Cell strains +JM	None	None	None

Calculation of viral inhibitory efficacy:
Substituting the mean of the three test results obtained the following results:

Influenza virus inhibition percentage = [1-10^ (-（5.1–3.2）)] x 100 = **98.74**

Conclusion

The experiment results show that a 0.625% concentration of the JM nanomaterials inhibit cellular infection of influenza A virus. The percentage of viral inhibition was **98.74%**.