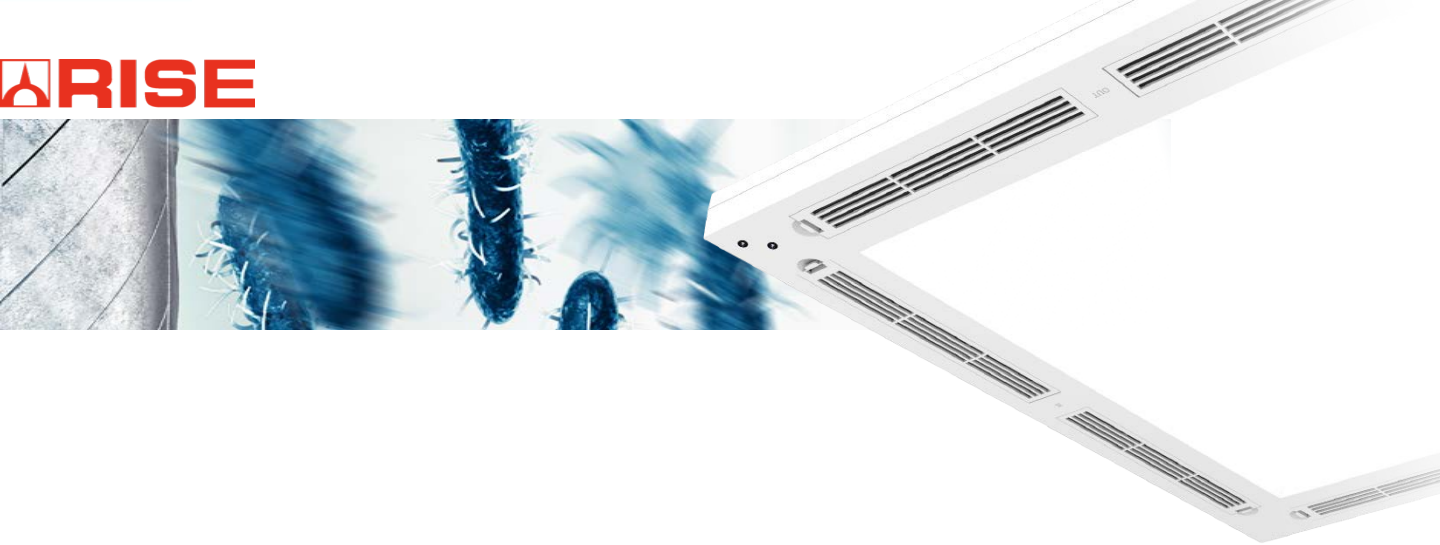

V-Buster

Chapter of TTA (NTDA)

(Nanocomposite Material)

呼吸道融合病毒

Respiratory Fusion Virus

& H1N1 測試報告

國泰綜合醫院
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JM 奈米新型複合材料抑制呼吸道融合病毒感染細胞能力之測試結果報告

測試試劑

JM 奈米新型複合材料

計畫委託

京程科技股份有限公司

計畫執行單位

醫學研究部臨床醫學研究中心細胞生物研究室

測試實驗室

汐止國泰綜合醫院病毒實驗室

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計畫摘要

計畫名稱：JM 奈米新型複合材料抑制呼吸道融合病毒感染細胞能力之測試

實驗設計：本計畫就 JM 奈米材料於病毒懸浮液中對呼吸道融合病毒抑制作用進行實驗室測試。使用 $TCID_{50}$ 方法進行抗病毒測試，觀察經 JM 材料作用後之病毒培養液中被感染細胞的細胞病變效應推算抑制病毒能力。

測試目的試劑：JM 奈米新型複合材料

試劑提供：京程科技股份有限公司

桃園縣龜山鄉民生北路一段 40-2 號 5 樓之 3

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測試內容

實驗材料

病毒株來源

Respiratory Syncytial Virus (RSV)，來自於美國病理學會能力試驗病毒株。

宿主細胞

Vero 細胞株（BCRC 60013），購自生物資源保存及研究中心。

實驗方法

甲、 細胞培養

1. 將 Vero 細胞株接種於 24 孔培養盤。
2. 以 Minimum Essential Medium（MEM）+8% Fetal Bovine Serum（FBS），36℃、5%CO_2 培養 48 小時至生長全滿。
3. 丟棄培養液，以 Phosphate Buffer Saline（PBS）沖洗 2 次備用。

乙、 病毒製備

1. 將病毒接種於含有細胞株之培養管。

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2. 以 MEM，36℃、5%CO_2 培養至 48 小時至生成細胞病變。

3. 刮除細胞，離心 6000 rpm， 2 分鐘。

4. 吸取上清液即為病毒懸浮液。

5. 將 100 uL 病毒懸浮液加入 900 uL MEM，進行 10 倍稀釋。

6. 將 100 uL 稀釋液加入 900 uL MEM，進行連續 10 倍稀釋。

編號	1	2	3	4	5	
MEM	900	900	900	900	900	
病毒液	100	0	0	0	0	
序列稀釋		100	100	100	100	100
最終體積	900	900	900	900	900	
最終濃度	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	

丙、 病毒 $TCID_{50}$ 測試

對照組

24 孔培養盤的細胞株，第 1 欄 4 孔不接種為細胞株品管。第 2-6 欄腸病毒分別接種 10 倍、10^2 倍、10^3 倍、10^4 倍、10^5 倍稀釋之病毒懸浮液 200 uL。

實驗組

1. 將 100 uL 病毒懸浮液加入 400 uL MEM，進行 5 倍

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稀釋。

2. 將 50 uL 稀釋液加入 450 uL MEM，進行連續 10 倍稀釋。

3. 配製 1.25%消毒劑（75 uL 消毒劑+5925 uL MEM），上述每個稀釋液加入 450 uL。

4. 另外製備一個 1.25%消毒劑 450 uL 加入 MEM 450 uL，不含病毒，是為 JM 毒性測試。

編號	BC	B1	B2	B3	B4	B5
MEM	450	400	450	450	450	450
病毒液	0	100	0	0	0	0
序列稀釋		50	50	50	50	50 丟棄 50
1.25%消毒劑	450	450	450	450	450	450
最終體積	900	900	900	900	900	900
病毒最終濃度	0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
消毒劑最終濃度	0.625%	0.625%	0.625%	0.625%	0.625%	0.625%

5. 室溫照 UV 一小時。

6. 24 孔培養盤的細胞株，第 1 欄 4 孔接種 JM 毒性測試，其餘 5 欄分別接種 10^1 倍、10^2 倍、10^3 倍、10^4 倍、10^5 倍稀釋之病毒懸浮液與 0.625% JM 作用後產物 200 uL。

實驗組與對照組均於 36℃、5%CO_2 感染一小時，期間每 20 分鐘搖動混合一次。以每孔加入 MEM，36℃、5%CO_2

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培養至 5 天，每天觀察細胞病變孔數。加入 4% Formaldehyde 1 mL，室溫靜置一小時，以自來水沖洗 2 次再加入 0.5% Crystal violet 1 mL，室溫靜置 5 分鐘。

丁、 判讀與計算

1. $TCID_{50}$ 之計算採用 Reed-Muench method。
2. 抑制病毒效能之計算公式：

抑制百分比= [1 - 10^ (-（對照組 Viral load (Log10TCID50) -實驗組 Viral load (Log10TCID50)）)] x 100

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測試結果

呼吸道融合病毒

Group	Viral load ($Log_{10}TCID_{50}$)		
	1st	2nd	3rd
病毒株	3.0	4.5	4.7
病毒株+JM	2.5	4.0	3.7
細胞株	None	None	None
細胞株+JM	None	None	None

計算抑制病毒效能：

以第三次實驗結果值帶入計算：

呼吸道融合病毒抑制百分比=[1-10^ (-（4.7 – 3.7）)]x100=**90.00**

結論

本次實驗結果顯示，0.625%濃度的 JM 材料具有抑制呼吸道融合病毒感染細胞之能力。經計算抑制能力可達 **90.00%**。

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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

Cheng-Yuan Tsai, Tsai-Yun Chu, Qing-Dong Ling

Principal Investigator

Qing-Dong Ling

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%**.