The Influence of Different Cell Culture Media on the Infectivity Yield of Dengue Viruses

Principal Investigators : Ananda Nisalak, M.D.
Robert McNair Scott, LTC, MC

Associate Investigators : Yeepu Keokarn
Naowayubol Nutkumhang

OBJECTIVE : To determine the effect of different cell culture media on the infectivity yields of dengue 1, 2, 3 and 4 viruses in LLC-Mk_2 cells.

BACKGROUND : Epidemiological, clinical and molecular investigations on dengue viruses continue to be complicated by low infectivity yields of these viruses in cell cultures and mice. The low yields are a major concern of the epidemiologist and clinician in regard to the isolation and identification of dengue viruses. Even with a virus isolation, repeated passage using in vitro systems may not raise the infectivity titers sufficiently to allow for identification by the plaque reduction neutralization test.

Recent findings suggested that the yield of dengue 2 virus in LLC-Mk_2 cells was greater when the cells were maintained on Roswell Park Memorial Institute (RPMI) 1640 medium as opposed to Medium-199 (M-199). The influence of 1640 medium on the replication of other dengue viruses is unknown; no investigations have been conducted to determine if RPMI 1640 medium will increase the possibility of isolating and identifying dengue viruses obtained from naturally infected mosquitoes or humans.

METHODS : Dengue viruses 1, 3 and 4 were isolated from patient's plasma. Dengue 2 virus was isolated from a mosquito captured in the house of a dengue hemorrhagic fever patient. Dengue 2 virus (BM-50-76) was in the first LLC-Mk_2 passage while the other viruses (dengue-1 CH2624-74, dengue-3, 1337-74, and dengue-4, VN7-73) each received 10 passages in LLC-Mk_2 cells. LLC-Mk_2 cells were propagated in one ounce bottles employing Medium-199 supplemented with 15% calf serum. Confluent cell monolayers, free of medium, were inoculated with 0.2 ml aliquots of selected dilutions of each virus; the cultures were then incubated at 35°C for 2 hours. The cultures were replenished with the desired medium and placed at 35°C for 6 days. Media employed included M-199, Minimal Eagle's Media (MEM) and RPMI 1640 with and without fetal calf serum (FCS). On day seven, post inoculation cultures were subjected to a freeze-thaw cycle and then assayed for virus employing standard plaque techniques in LLC-Mk_2 cells.

RESULTS : Dengue virus infectivity titers in LLC-Mk_2 employing different media are shown in Tables 1 to 4. Except for dengue 2 virus, which appeared to replicate best in the presence of RPMI 1640 medium, no apparent difference in the infectivity yield was associated with different media. However, for dengue 1, 2, and 4, the dose of virus required to initiate infection appeared to vary depending on the medium used. In all cases when the lowest multiplicity of infection (MOI) was employed, virus was detected only in LLC-Mk_2 cultures

maintained on RPMI 1640 medium. Also, the yield of virus appeared to be related to the MOI, that is, the optimum amount of inoculum tended to result in a satisfactory virus yield.

There were no marked differences in the infectivity yield of dengue virus in the presence of different media. The observation that minimal doses of dengue virus could initiate an infection in the presence of RPMI 1640 medium, suggests that this medium might increase the chances of isolating dengue viruses from biological specimens. Therefore, RPMI 1640 medium will be employed in place of M-199 for our standard viral isolation procedures.

Table 1. Dengue Type 1[a] Virus Infectivity Yield in LLC-Mk_2 Maintained on Different Media

Inoculum (PFU/0.2 ml)	Media					
	M-199	M-199 +5% FCS	MEM	MEM +5% FCS	RPMI 1640	RPMI 1640 +5% FCS
1×10^4 (TNTC)[b]	4×10^3[c]	1.6×10^4	3.0×10^4	1.1×10^4	1.6×10^4	1.1×10^4
1×10^3 (TNTC)	4×10^4	7.0×10^3	2.6×10^4	1.4×10^4	1.7×10^4	7.0×10^4
1×10^2 (38)	7×10^3	9.0×10^2	1.0×10^4	3.0×10^3	1.2×10^4	7.0×10^3
1×10^1 (9)	6×10^3	2.9×10^2	5.0×10^3	7.0×10^2	8.0×10^3	3.0×10^3
1 (1)	0[d]	0	0	0	2.1×10^2	1.2×10^2
0	0	0	0	0	0	0

[a] Dengue type 1, CH2624-74, Mk_2-10

[b] Actual count

[c] PFU/0.2 ml of seeds harvested at day 7

[d] Virus not detected

Table 2. Dengue Type 2[a] Virus Infectivity Yield in LLC-Mk_2 Maintained on Different Media

Inoculum (PFU/0.2 ml)	Media				
	M-199	M-199 +5% FCS	MEM	MEM +5% FCS	RPMI 1640
2×10^4 (TNTC)[b]	2×10^3[c]	6×10^3	3×10^3	4×10^3	8×10^5
2×10^3 (TNTC)	4×10^3	6×10^3	3×10^3	6×10^3	1×10^6
2×10^2 (24)	1×10^3	1×10^3	5×10^3	1×10^2	2×10^5
2×10^1 (2)	3×10^2	1×10^2	8×10	1×10^2	5×10^4
2	0[d]	0	0	0	5×10^3
0	0	0	0	0	0

[a] Dengue Type 2, BM50-76 Mk_2-1

[b] Actual count

[c] PFU/0.2 ml of seeds harvested at day 7

[d] Virus not detected

Table 3. Dengue Type 3[a] Virus Infectivity Yield in LLC-Mk_2 Cells Maintained on Different Media

Inoculum (PFU/0.2 ml)	Media					
	M-199	M-199 +5% FCS	MEM	MEM +5% FCS	RPMI 1640	RPMI 1640 +5% FCS
1.5×10^3 (TNTC)[b]	1×10^4[c]	2×10^3	7.2×10^4	7×10^4	3.8×10^6	5×10^5
1.5×10^2 (45)	1.8×10^4	5×10^3	2×10^5	2×10^4	3.8×10^6	1×10^6
1.5×10^1 (10)	2×10^3	2×10^3	2×10^4	2.9×10^4	4×10^4	1×10^4
1.5 (1.5)	7×10^1	1.8×10^1	6×10^2	8×10^2	1.6×10^4	7×10^4
0	0[d]	0	0	0	0	0

[a] Dengue Type 3, 1337-74, Mk_2-10

[b] Actual count

[c] PFU/0.2 ml of seeds harvested at day 7

[d] Virus not detected

Table 4. Dengue Type 4[a] Virus Infectivity Yield in LLC-Mk_2 Cell Maintained on Different Media

Inoculum (PFU/0.2 ml)	Media					
	M-199	M-199 +5% FCS	MEM	MEM +5% FCS	RPMI 1640	RPMI 1640 +5% FCS
1×10^4 (TNTC)[b]	1.6×10^4[c]	2.0×10^3	8.0×10^4	2.0×10^4	1.0×10^4	3.0×10^4
1×10^3 (TNTC)	1.2×10^4	4.0×10^3	2.0×10^4	1.0×10^4	2.0×10^4	1.2×10^4
1×10^2 (26)	1.0×10^4	1.5×10^1	7.0×10^2	1.0×10^4	1.0×10^4	5.1×10^4
1×10^1 (3)	3.0×10^1	1.2×10^3	3.0×10^3	2.0×10^4	1.3×10^4	2.4×10^4
1 (1)	0[d]	2.0×10^0	0	0	3.1×10^2	1.8×10^2

a Dengue Type 4, VN-H7-73, Mk_2-10

b Actual count

c PFU/0.2 ml of seeds harvested at day

d Virus not detected