

CHAPTER V

RESULTS

Propagation of virus from clinical isolates

Total of 121 specimens collected from January 1998 to June 2002 were HSV culture positive by SVC. They were all subcultured in Vero cells to increase the viral titer for further studies. Among those, only 86 isolates (71.07%; 86/121) were successfully propagated. Some of them either could not be propagated to yield high titers in Vero cell culture or were lost during subcultivation. The number of passages varied from the second to the sixth. The results were shown in Table 4.

Typing of HSV isolates

To type HSV isolates, an indirect IFA using MAb HSV type specific was done as described in Materials and Methods. A number of 86 isolates were determined. The characteristic of immunofluorescent staining patterns of HSV infected cells were shown in Figure 6. Three examples of clinical isolates were demonstrated. No.42/2001 was HSV-1, and No.10/1999 was HSV-2 while No.11/2002 was found to be both HSV-1 and HSV-2, suggesting there was mixed infection of both types in the patients.

Total of 86 clinical isolates from both genital and non-genital were typed by indirect IFA using MAb HSV type specific and the results were shown in Table 5. Among these clinical samples, HSV-1 was found predominately 62.79% (54/86), followed by HSV-2 34.88% (30/86) and only 2.32% (2/86) were mixed infection (Table 6). When the samples were divided according to the site of infection, non-genital specimens with HSV-1 and HSV-2 were detected in 90.91% (20/22), and 9.09% (2/22), respectively. No mixed infection was found. In genital specimens, HSV-1 was detected 53.12% (34/64), 43.75 % (28/64) of HSV-2, and 3.12% (2/64) of mixed infection were shown (Table 7).

Table 4: The number of HSV propagation which were positive for HSV isolating during January 1998 to June 2002.

Period	Total of positive HSV specimen	The number of passages of propagated HSV isolates					Total of successfully propagated HSV
		P ₂	P ₃	P ₄	P ₅	P ₆	
JAN-DEC 1998	10	1	2	-	-	-	3
JAN-DEC 1999	21	-	13	1	-	-	14
JAN-DEC 2000	25	-	13	1			14
JAN-DEC 2001	48	26	5	5	3	5	44
JAN-JUNE 2002	17	7	4	-	-	-	11
Total	121 (100%)	-	-	-	-	-	86 (71.07%)

P = Passage

Figure 6: Immunofluorescence staining patterns of HSV-infected cells. Standard HSV-1 (KOS), standard HSV-2 (Baylor 186), and 3 clinical isolates were shown (magnification 40x).

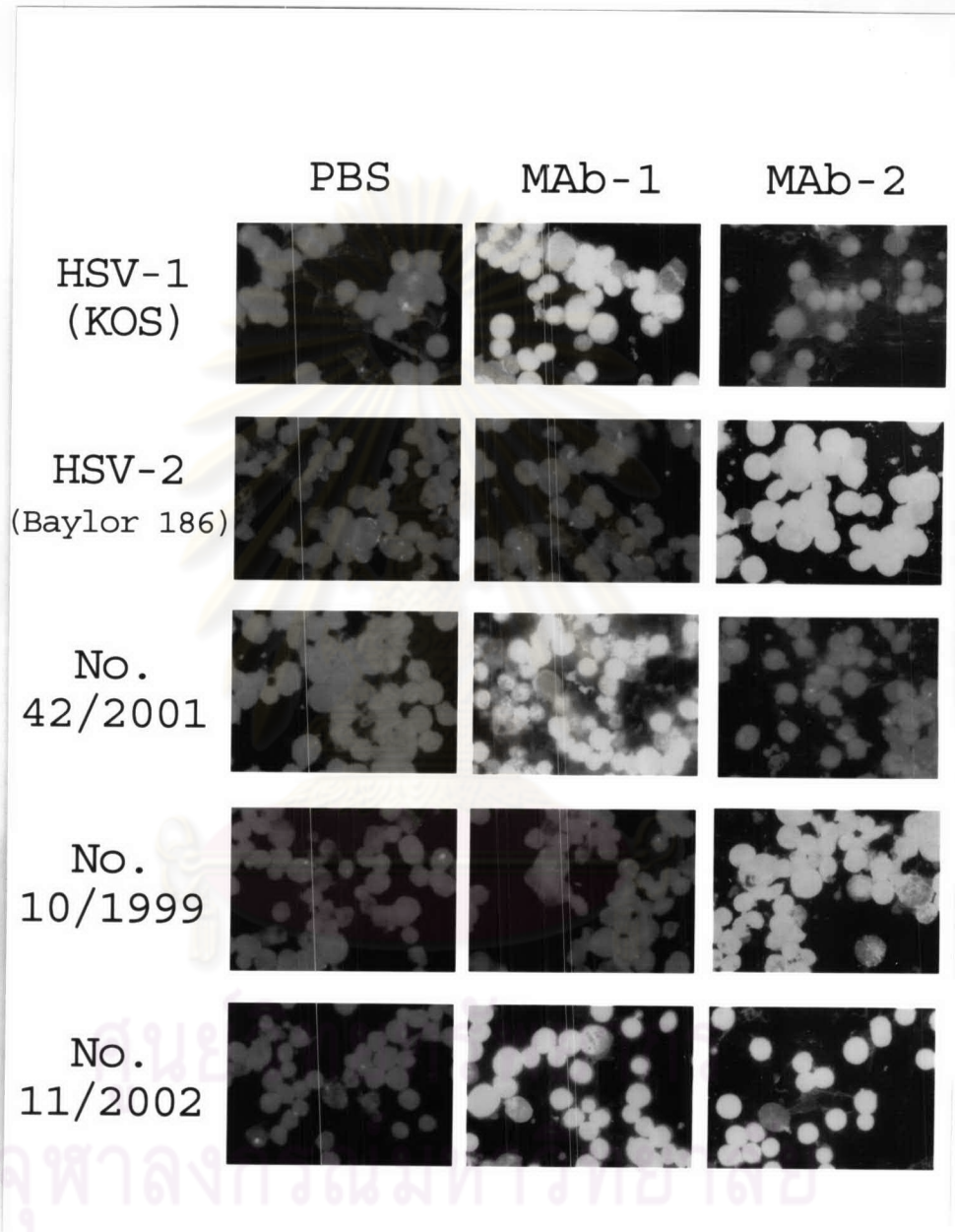


Table 5: The data of 86 tested clinical specimens typing by indirect IFA using MAb HSV-type specific antibodies.

Period	No.	Sex	Site of collected HSV specimens	HSV type
JAN-DEC 1998 (n=3)	1/1998	M	NG	HSV-1
	2/1998	M	NG	HSV-1
	3/1998	M	NG	HSV-1
JAN-DEC 1999 (n=14)	1/1999	F	NG	HSV-1
	2/1999	F	G	HSV-2
	3/1999	F	NG	HSV-1
	4/1999	M	G	HSV-1
	5/1999	F	G	HSV-2
	6/1999	F	G	HSV-2
	7/1999	M	G	HSV-1
	8/1999	F	G	HSV-2
	9/1999	F	G	HSV-1
	10/1999	F	G	HSV-2
	11/1999	F	NG	HSV-1
	12/1999	F	G	HSV-2
	13/1999	F	G	HSV-1
	14/1999	F	G	HSV-1

Period	No.	Sex	Site of collected HSV specimens	HSV type
JAN-DEC 2000 (n=14)	1/2000	F	G	HSV-2
	2/2000	F	G	HSV-2
	3/2000	M	NG	HSV-1
	4/2000	M	G	HSV-1
	5/2000	F	G	HSV-1
	6/2000	M	NG	HSV-2
	7/2000	M	NG	HSV-2
	8/2000	F	G	HSV-2
	9/2000	M	NG	HSV-1
	10/2000	F	NG	HSV-1
	11/2000	F	G	HSV-2
	12/2000	F	G	HSV-1
	13/2000	M	NG	HSV-1
	14/2000	F	G	HSV-1&-2
JAN-DEC 2001 (n=44)	1/2001	F	G	HSV-1
	2/2001	F	G	HSV-1
	3/2001	F	G	HSV-1
	4/2001	M	NG	HSV-1

Period	No.	Sex	Site of collected HSV specimens	HSV type
JAN-DEC 2001 (n=44)	5/2001	F	G	HSV-2
	6/2001	F	G	HSV-2
	7/2001	F	G	HSV-2
	8/2001	F	G	HSV-2
	9/2001	F	G	HSV-2
	10/2001	F	G	HSV-2
	11/2001	F	G	HSV-1
	12/2001	F	G	HSV-1
	13/2001	M	NG	HSV-1
	14/2001	M	NG	HSV-1
	15/2001	F	G	HSV-1
	16/2001	F	G	HSV-1
	17/2001	F	G	HSV-2
	18/2001	F	G	HSV-1
	19/2001	F	G	HSV-1
	20/2001	F	G	HSV-1
	21/2001	F	G	HSV-1
	22/2001	F	G	HSV-1

Period	No.	Sex	Site of collected HSV specimens	HSV type
JAN-DEC 2001 (n=44)	23/2001	F	G	HSV-1
	24/2001	F	NG	HSV-1
	25/2001	F	G	HSV-1
	26/2001	F	G	HSV-2
	27/2001	F	G	HSV-2
	28/2001	F	G	HSV-1
	29/2001	F	G	HSV-2
	30/2001	F	G	HSV-2
	31/2001	F	G	HSV-1
	32/2001	F	G	HSV-1
	33/2001	F	G	HSV-1
	34/2001	F	G	HSV-1
	35/2001	F	G	HSV-1
	36/2001	F	G	HSV-2
	37/2001	M	G	HSV-1
	38/2001	F	G	HSV-1
	39/2001	M	G	HSV-1
40/2001	F	NG	HSV-1	

Period	No.	Sex	Site of collected HSV specimens	HSV type
JAN-DEC 2001 (n=44)	41/2001	F	NG	HSV-1
	42/2001	F	NG	HSV-1
	43/2001	F	NG	HSV-1
	44/2001	F	NG	HSV-1
JAN-JUNE 2002 (n=11)	1/2002	F	G	HSV-2
	2/2002	F	G	HSV-2
	3/2002	F	G	HSV-1
	4/2002	F	G	HSV-2
	5/2002	F	G	HSV-1
	6/2002	F	G	HSV-2
	7/2002	F	G	HSV-1
	8/2002	F	G	HSV-2
	9/2002	F	NG	HSV-1
	10/2002	F	G	HSV-2
	11/2002	F	G	HSV-1&-2

F = Female M = Male

NG = Non genital G = Genital

n = Number of Sample

Table 6: Summarized typing data of clinical specimens in each years (January 1998- June 2002). The information of gender was indicated.

Period	No. of specimens	SEX	HSV-1	HSV-2	HSV-1&-2
JAN-DEC 1998	3	F	-	-	-
		M	3	-	-
JAN-DEC 1999	14	F	6	6	-
		M	2	-	-
JAN-DEC 2000	14	F	3	4	1
		M	4	2	-
JAN-DEC 2001	44	F	27	12	-
		M	5	-	-
JAN-JUNE 2002	11	F	4	6	1
		M	-	-	-
Total	86 (100%)	-	54 (62.79%)	30 (34.88%)	2 (2.32%)

F = Female

M = Male

No. = Number

Table 7: The distribution of HSV type due to site of infection and gender.

Sex	No. of Specimens	Non-genital lesion (n=22)(100%)			Genital lesion (n=64)(100%)		
		HSV-1	HSV-2	HSV-1&2	HSV-1	HSV-2	HSV-1&2
F	70	11	0	-	29	28	2
M	16	9	2	-	5	0	-
Total	86	20 (90.90%)	2 (9.09%)	-	34 (53.12%)	28 (43.75%)	2 (3.12%)

F = Female

M = Male

No. = Number

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Antiviral susceptibility testing

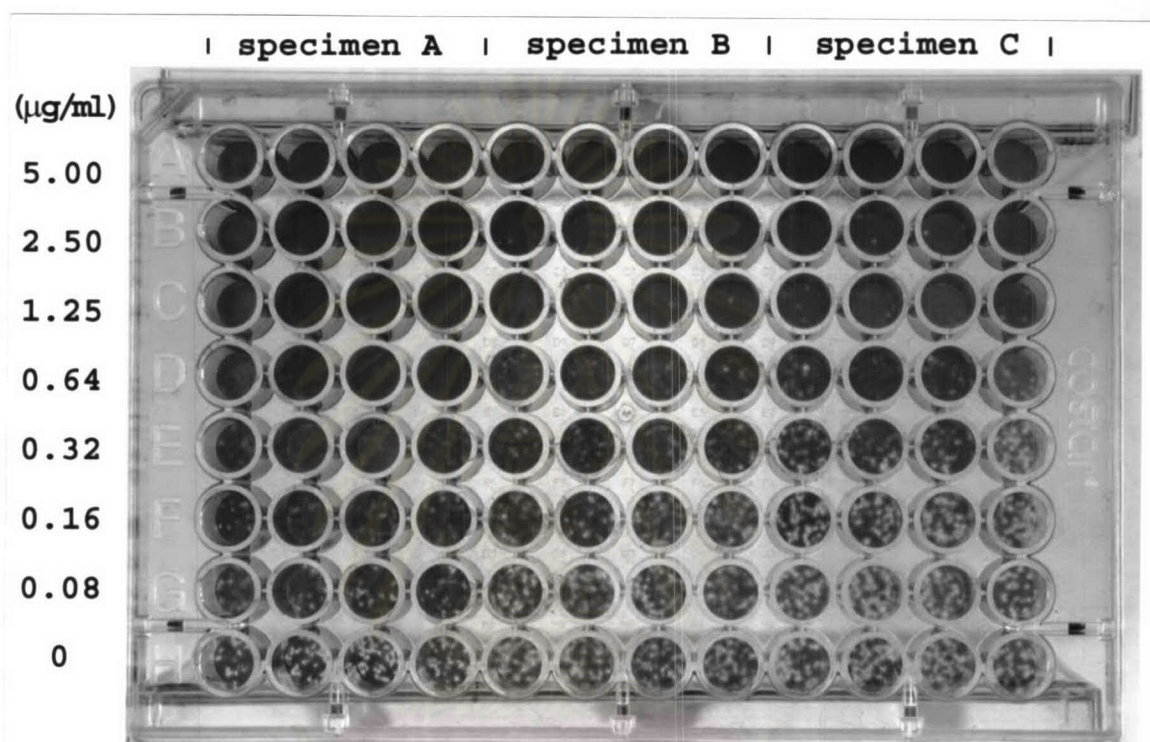
To determine the sensitivity of HSV isolate to ACV by PRA, various concentrations of ACV were tested i.e., 5, 2.5, 1.25, 0.62, 0.31, 0.16, 0.08, and 0 $\mu\text{g/ml}$. Each experiment was done in quadruplicate well (Figure 7). Plaques were counted and IC_{50} was calculated from linear equation. HSV isolates were considered susceptible to ACV when the IC_{50} was $< 3 \mu\text{g/ml}$ and resistant when the IC_{50} was $\geq 3 \mu\text{g/ml}$ (127). The standard HSV-1 strain KOS and HSV-2 strain Baylor 186 were run parallel in each assay.

Only 80 HSV isolates were assayed for ACV susceptibilities. Two isolates with mixed infection were excluded. The other four isolates lost during propagation to get high titer of viruses. The results were shown in Table 8 and 9 as IC_{50} of each HSV type. Scattering graph of IC_{50} values were plot for observing dispersion of the data [Figure 8 (HSV-1) and Figure 9 (HSV-2)].

The range of IC_{50} of both HSV-1 and HSV-2 isolates were presented in Table 10 and Figure 10. The range of IC_{50} of 52 HSV-1 isolates was 0.07-0.97 $\mu\text{g/ml}$ and 28 HSV-2 isolates were 0.17-1.66 $\mu\text{g/ml}$. The mean IC_{50} of ACV for HSV-1 and HSV-2 isolates were 0.36 $\mu\text{g/ml}$ (SD: 0.23) and 0.54 $\mu\text{g/ml}$ (SD: 0.36) while that of HSV-1 (KOS) and HSV-2 (Baylor 186) were 0.48 (SD: 0.11) and 0.58 (SD: 0.04), respectively. There was statistically significant difference between IC_{50} of HSV-1 and HSV-2 isolates ($p = 0.02$).

In this study, no ACV^r HSV was found in HSV isolates which means the prevalence of ACV^r HSV was zero. Therefore, characterization of TK-mutation in ACV^r HSV by DNA sequencing method was omitted.

Figure 7: Assay of sensitivity to ACV of HSV isolates by PRA in 96 well-plate. Various concentrations of ACV were tested i.e., 5, 2.5, 1.25, 0.62, 0.31, 0.16, 0.08, and 0 $\mu\text{g/ml}$ and done in quadruplicated wells for each concentration.



ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 8: The IC₅₀ values of HSV-1 isolates and standard HSV-1 (strain KOS).

Period	No. of HSV-1 isolates	IC ₅₀ of HSV-1 isolates (µg/ml)
JAN-DEC 1998 (n=3)	1/1998	0.20
	2/1998	0.23
	3/1998	0.13
JAN-DEC 1999 (n=8)	1/1999	0.07
	3/1999	0.63
	4/1999	0.37
	7/1999	0.49
	9/1999	0.39
	11/1999	0.28
	13/1999	0.97
	14/1999	0.86
JAN-DEC 2000 (n=7)	3/2000	0.23
	4/2000	0.43
	5/2000	0.10
	9/2000	0.49
	10/2000	0.15
	12/2000	0.24
	13/2000	0.25

Period	No. of HSV-1 isolates	IC ₅₀ of HSV-1 isolates (µg/ml)
JAN-DEC 2001 (n=30)	1/2001	0.23
	2/2001	0.36
	3/2001	0.36
	4/2001	0.24
	11/2001	0.67
	12/2001	0.29
	13/2001	0.07
	14/2001	0.08
	15/2001	0.10
	16/2001	0.29
	18/2001	0.12
	20/2001	0.15
	21/2001	0.20
	22/2001	0.14
	23/2001	0.09
24/2001	0.20	
25/2001	0.33	
31/2001	0.41	

Period	No. of HSV-1 isolates	IC ₅₀ of HSV-1 isolates (µg/ml)
JAN-DEC 2001 (n=30)	32/2001	0.63
	33/2001	0.27
	34/2001	0.56
	35/2001	0.71
	37/2001	0.26
	38/2001	0.18
	39/2001	0.89
	40/2001	0.16
	41/2001	0.41
	42/2001	0.65
	43/2001	0.83
44/2001	0.28	
JAN-JUNE 2002 (n=4)	3/2002	0.34
	5/2002	0.57
	7/2002	0.29
	9/2002	0.61

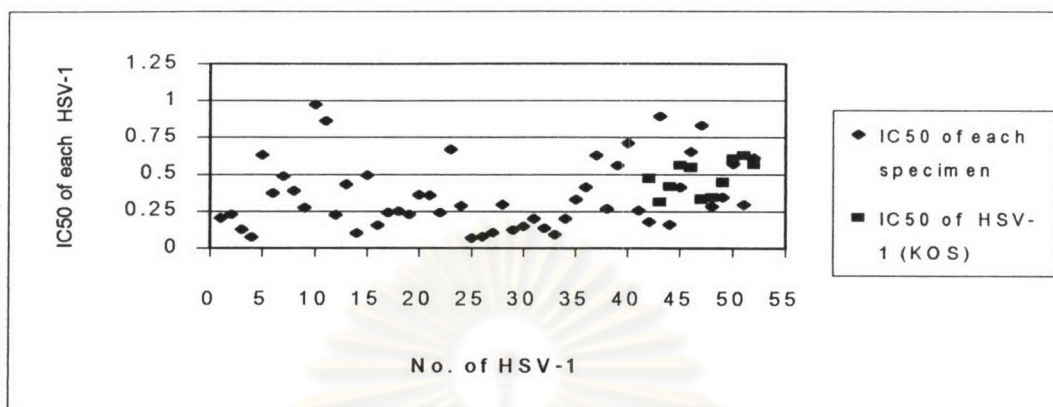
Period	No. of HSV-1 isolates	IC ₅₀ of HSV-1 isolates (µg/ml)
	Standard	0.48
	HSV-1	0.31
	(strain KOS)	0.42
		0.56
		0.55
		0.33
		0.34
		0.45
		0.60
		0.63
		0.57

No. = Number of HSV-1

IC₅₀ = 50 % of inhibitory concentration

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Figure 8: Scatter plot of IC_{50} value of HSV-1 isolates and standard HSV-1 strain KOS.



No. = Number of HSV-1

IC_{50} = 50 % of inhibitory concentration

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 9: The IC₅₀ values of HSV-2 isolates and standard HSV-2 (strain Baylor186).

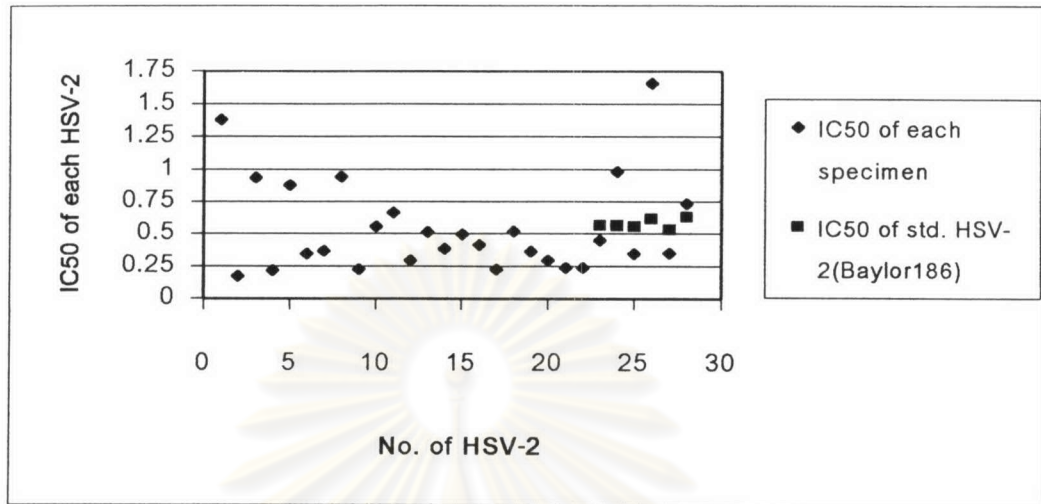
Period	No. of HSV-2 isolates	IC ₅₀ of HSV-2 isolates (µg/ml)
JAN-DEC 1999 (n=6)	2/1999	1.38
	5/1999	0.17
	6/1999	0.94
	8/1999	0.22
	10/1999	0.88
	12/1999	0.35
JAN-DEC 2000 (n=6)	1/2000	0.37
	2/2000	0.94
	6/2000	0.22
	7/2000	0.56
	8/2000	0.66
	11/2000	0.30
JAN-DEC 2001 (n=11)	5/2001	0.51
	6/2001	0.38
	7/2001	0.50
	8/2001	0.41
	9/2001	0.22
	10/2001	0.52

Period	No. of HSV-2 isolates	IC ₅₀ of HSV-2 isolates (µg/ml)
JAN-DEC 2001 (n=11)	26/2001	0.36
	27/2001	0.29
	29/2001	0.24
	30/2001	0.24
	36/2001	0.45
JAN-JUNE 2002 (n=5)	1/2002	0.98
	2/2002	0.34
	4/2002	1.66
	6/2002	0.35
	8/2002	0.73
	Standard	0.57
	HSV-2	0.56
	(Baylor 186)	0.56
		0.62
		0.54
	0.63	

No. = Number of HSV-2

IC₅₀ = 50 % of inhibitory concentration

Figure 9: Scatter plot of IC_{50} value of HSV-2 isolates and standard HSV-2 strain Baylor 186.



No. = Number of HSV-2

IC_{50} = 50 % of inhibitory concentration

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 10: The susceptibility results of either HSV-1 or HSV-2 isolates to ACV.

Type		Range of IC ₅₀ ($\mu\text{g/ml}$)	Mean \pm SD of IC ₅₀
HSV-1	isolates (n=52)	0.07-0.97	0.36 \pm 0.23
	Strain KOS(n=11)	0.31-0.63	0.48 \pm 0.11
HSV-2	isolates (n=28)	0.17-1.66	0.54 \pm 0.36
	Strain Baylor186(n=6)	0.54-0.63	0.58 \pm 0.04

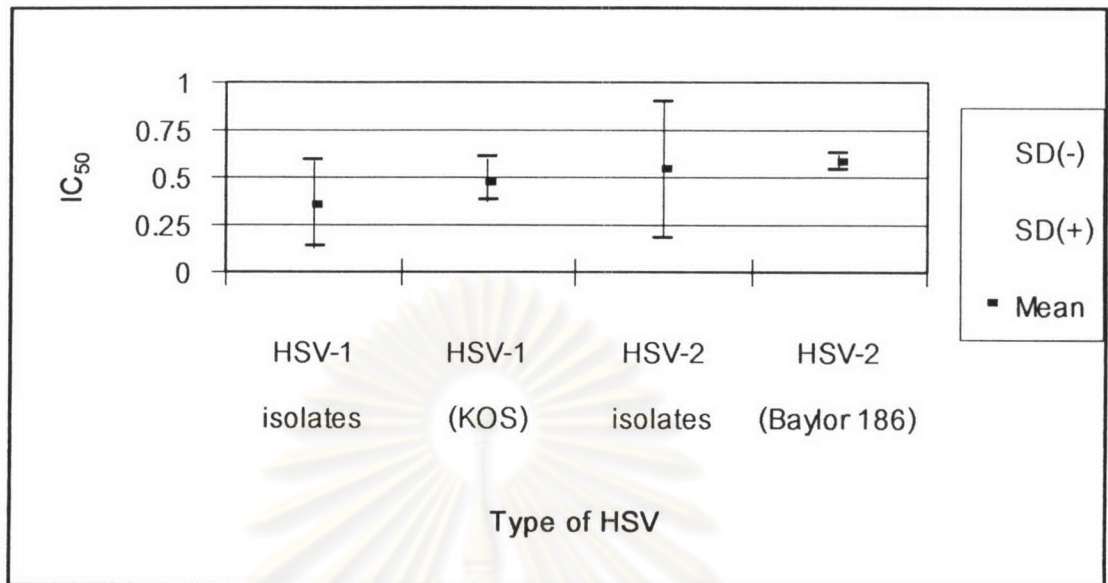
n = Number of sample

SD = Standard deviation

IC₅₀ = 50 % of inhibitory concentration

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Figure 10: Mean \pm SD of IC₅₀ of HSV-1 and HSV-2 viruses.



IC₅₀ = 50 % of inhibitory concentration

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย