Deletions and Duplication in Internal Inverted Repeat Sequence of Long Region/Unique Sequence of Long Region (IRL/UL) of Herpes Simplex Virus Type-i (HSV-i) Genome are not Evidently Associated with Intracranial and Foot-Pad Pathogenicity in Mouse Model

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Fazil Junejo (Pakistan Medical Research Council, Central Research Centre, National Institute of Health, Islamabad.)
S. Moira Brown (MRC Virology Unit, Institute of Virology*, Church Street, Glasgow, G11 5JR, United Kingdom.)

Abstract

The biological properties of three deletion variants (1704, 1705 and 1706) of herpes simplex virus type-1 (HSV-I) strain 17 syn+, were studied by establishing a base line pathogenicity of nine individual plaques from the parental 17 syn+ elite stock. Restriction enzyme analysis of deoxyribonucleic acid (DNA) horn each of the nine plaque stocks and intracranial inoculation into three weeks old BALB/C mice showed no difference in the size of fragments and distribution of the sites or their 50% lethal dose (LD50) values [plaque forming units (pfu)/mousel as compared to the parental 17 syn+ stock. Inoculation of the variants into three weeks old BALB/c mice showed that 1705 was not different in pathogenicity from the wild type following intracranial and footpad inoculations. On the other hand variants 1704 and 1706, when compared to the wild type virus were less virulent on intracranial inoculation i.e. the difference in LD5O values was approximately one log and two logs respectively and both the variants failed to kill any of the animals following footpad inoculation even at the dose of 1x107 pfu/mouse. During in vivo replication experiment in the peripheral nervous system of mice, 1704 and 1706 grew very poorly (JPMA 45:95,1995).

Introduction

Herpes simplex virus (HSV) is a neurotropic virus. In humans, invasion of the nervous system frequently leads to latent infection in sensory ganglia and in rare circumstances to fatal encephalitis[1,2]. The virus host relationship is highly complex and influenced by the genotype of both the virus and host. Many host factors have been shown to influence virulence including route of inoculation[3], age[4] and strain of the experimental animal[5]. Virus strains[6] and serial passage of virus in viv[7] and in vitro[8] may also effect the virulence. Individual strains of HSV differ in their level of neurovirulence[6,9]. Specific viral factors required for the virus replication at the periphery and for spread to the central nervous system may be the underlying cause of heterogeneity of individual virus strains. Viral factors playing a part in neuroinvasiveness have also been reported[7,10]. Heterogeneity in the neurovirulence of plaque purified stocks of the HSV-2 strain HG52 has been documented[11]. Although detectable deletions in the genome of the elite stock of HSV-2 strain HG52 were found at a frequency of 24%[12]. DNA profiles of the individual stocks inoculated intracranially had no obvious differences compared to wild type stock[11]. The variation affecting the phenotype of the virus was perhaps due to minor sequence alterations undetectable by conventional restriction enzymes methods. The neuropathogenic determinant of both HSV-l and 2 lies in inverted repeat sequences of long region (RL) between the ‘a’ sequence and immediate early 1 (IE1) gene. Loss of such sequence resulted ma non-nëurovirulent phenotyp[11,13]. The sequences carry, gene [infected cell polypeptide 34.5 Kd (ICP 34.5)] which lies in

the DR1 element (20 bp element present as direct repeat at the ends of ‘a’ sequence) of the ‘a’ sequence[14]. The product of this gene appears to be responsible for the HSV replication in mouse central nervous system[11]. A gene has also been found in African swine fever virus having striking similarity with neurovirulence associated gene (ICP 34.5) of herpes simplex virus[15]. We have investigated neurovirulence and peripheral nervous system replication efficiency of three spontaneously derived deletion variants (1704, 1705 and 1706) of HSV-1 strain 17 syn+. The sequence analysis and precise end points of the deletion have already been described[16]. It was further interesting to elaborate comparative analysis of the functions of HSV-l genes related to neuropathogenicity in the deletion variants.

Materials, Methods and Results

Isolation of single plaque stocks

To determine the basis for evaluating neurovirulence, intracranial inoculation of single plaque stocks picked from HSV-1 strain 17 syn+ elite stock were performed. The stock was plated onbaby hamster kidney 21 clone 13 (BHK-2 1 C13) cells and overlaid with methyl cellulose. Cell monolayer with the fewest plaques were washed twice with phosphate buffered saline (PBS)/call serum. Individually separated plaques were picked into 500 pfu PBS/call serum, sonicated and were purified an additional three times prior to further analysis: Nine well separated plaques were picked after washing thoroughly with PBS. High litre virus was propagated with one further passage inBHK-2 1 C13 cells. All the plaque deoxyribonucleic acids (DNAs) were in vivo radio-labelled with 5 micro Cunes (uCI) of 32P-orthophosphate for 48 hrs. and subjected to restriction endonuclease digestion with XbaI and HpaI[17]. As a representative sample, the profiles of five of these are shown in Figure 1.

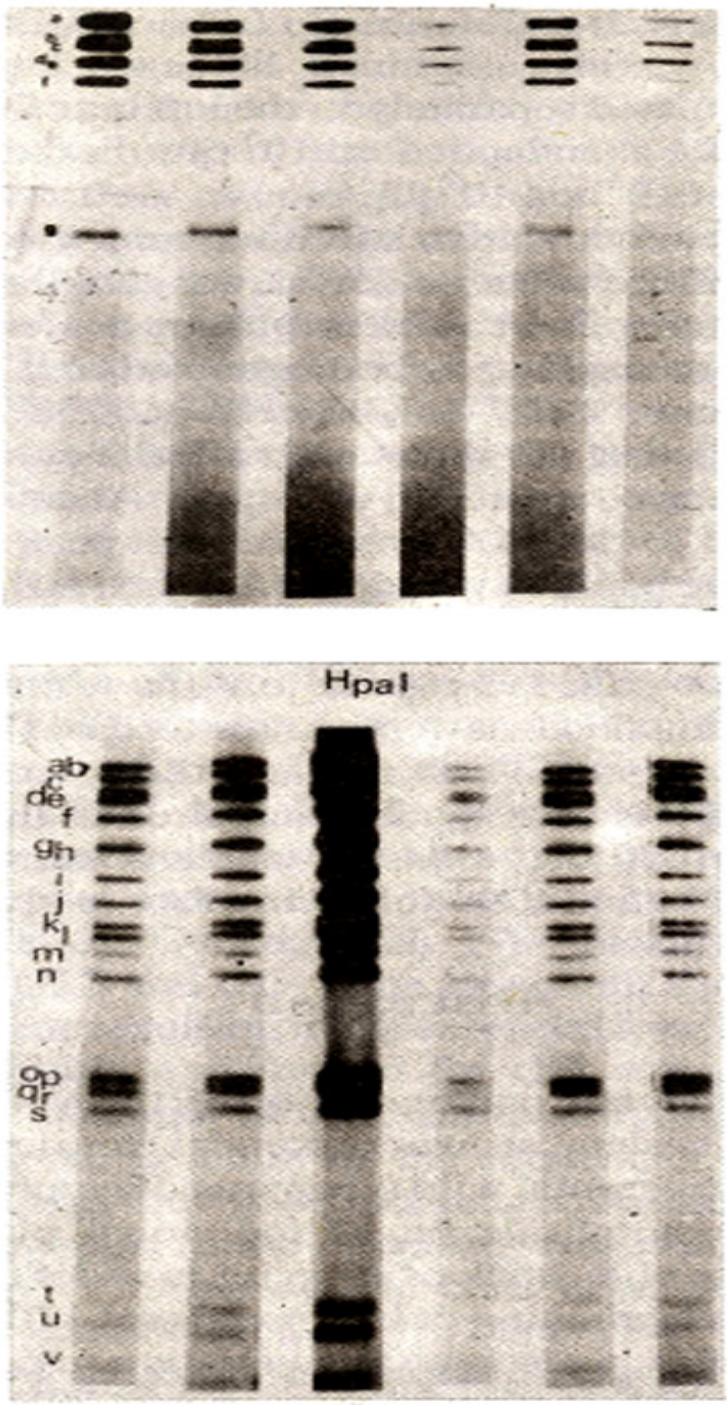

Figure 1. Autoradiograph of *Xba*I (a) and *Hpa*I (b) digests of viral DNA ^{32}P labelled *in vivo* of individual plaque stock of 17 syn+ (from left to right 17 syn+, Plaque Nos. 1, 2, 3, 4 and 5). Letters refer to specific fragments. The DNA products were separated on a 0.5% and 0.8% agarose gel in 'a' and 'b' respectively.

The result shows no apparent difference in the size of the fragments and distnbution of sites in the DNA of individual plaque stocks.

Neurovirulence of single plaque stocks of HSV-1 strain 17syn+

The nine plaques were separately inoculated intracrarnally into three weeks old BALB/c mice. Groups of four to eight mice were inoculated with a single dilution of each virus stock between 1-10^2 pfu/animal. The virus stocks were titrated on BHK-21 C13 cells on the day of inoculation, to determine

the precise quantity of virus inoculated. Mice were observed daily for 21 days post-inoculation and their clinical state recorded. The 50% lethal dose value (LD50) was calculated according to the formula of Reedand Muench[18] on the basis of deaths between days three and twenty one. The result shows that each plaque stock has an LD^{50} similar to the elite parental 17 syn+ stock (Table I).

Table I. LD_{50} values after intracranial inoculation of individual plaque stocks of $17\,syn^{+}$.

Plaque No.	1pfu*	$1x10^1$	$1x10^2$	LD_{50} pfu/mouse
1	0/5**	1/5	4/5	30.2
2	0/5	5/5	5/53.2	
3	1/5	2/5	5/5	15
4	0/5	2/5	5/5	14
5	5/5	5/5	5/5	<1
6	5/5	5/5	5/5	<1
7	0/5	2/5	3/5	30.2
8	1/5	4/5	5/5	3.2
9	3/5	4/5	5/5	<1

*Dose pfu/animal.
**Number of deaths/number of animals inoculated.
3 week old BALB/c mice were used.
0.025 ml of virus inoculated.

There is therefore, no apparent neurovirulence heterogeneity within the elite stock ofHSV-1 l7syn+.

Virulence analysis of the variants 1704, 1705 and 1706

To determine the pathogenic potential of the deletion variants, intracranial and foot-pad inoculations of the viruses were carried out in three weeks old BA.LB/c mice.

Intracranial inoculation

Groups of 5 to 10, three weeks old BALB/c mice were inoculated with $1\text{-}10^5$ pfu/mouse ofvariants. 0.025 ml of each dilution was inoculated intracranially into the left cerebral hemisphere after anaesthesia. The LD50 values of the variant compared to the wild type virus are shown in Table II.

Table II. LD_{50} values (pfu/mouse) after intracranial and foot pad inoculation of 17 syn+, 1704, 1705 and 1706.

Virus	Intracranial	Foot pad
17+	10	$2x10^5$
1704	$2x10^2$	$>1x10^7$
1705	11	$9x10^4$
1706	$4.6x10^3$	$>1x10^7$

3 week old BALB/c mice were used.
0.025 ml of virus inoculated.

Itcanbe seen that 17 syn+, 1704, 1705 and 1706 had an LD50 of 10, $2x10^2$, 11 and $4.6x\ 10^3$ pfu/mouse respectively. The variant 1705 was therefore not different from 17 syn+ but 1704 and 1706 were one log and two logs less neurovirulent respeclively. To determine whetherthe differences inthe LD50 values of the virus stocks were related to the number of virus particles within the stocks, particle counts were also performed. 17 syn+, 1704, 1705 and 1706 had particle pfu ratios of 72, 64,46 and 125 respectively. This shows that all the virus stocks had the ratios within the acceptable ranges of HSV-1.

Foot-pad inoculation

To determine the general pathogenicity of the variants, peripheral inoculation via the foot pad of BALB/c mice was carried out. In this system 0.025 nil of serially diluted virus from 10^2 to 10^7 pfu was inoculated in the left rear foot-pad of three weeks old BALB/c mice. Table II shows that 17 syn+ and 1705 are comparable with LD50 values of approximately 10^5 pfu/mouse. Doses of 10^7 pfu/mouse of 1704 and 1706 were not able to kill any of the mice. It was not possible to infect witha dose higher than 10^7 pfu/mouse.

Replication efficiencies of 1704 and 1706 in vivo

The possibility, that differential abilities to replicate in the mouse peripheral nervous system were responsible for causing the observed differences in LD50 values of 17 syn+, 1704 and 1706, was also examined. Three weeks old BALB/c mice were inoculated separately in the left rear foot -pad with 17 syn+ and 1706 at an input dose 10^5 pfu/mouse and 1704 at aninputdoses of 10^5 and 10^7 pfu/mouse. AtO, 24,72,96, 120 and 144 hr post infection two surviving mice from each time point were sacrificed and nine dorsal root ganglia(DRG) (one thoracic, six lumbar and two sacral) from the left side of the spinal cord were explanted, homogenized and assayed for virus by titration on BHK-21 C13 cells at 37°C.

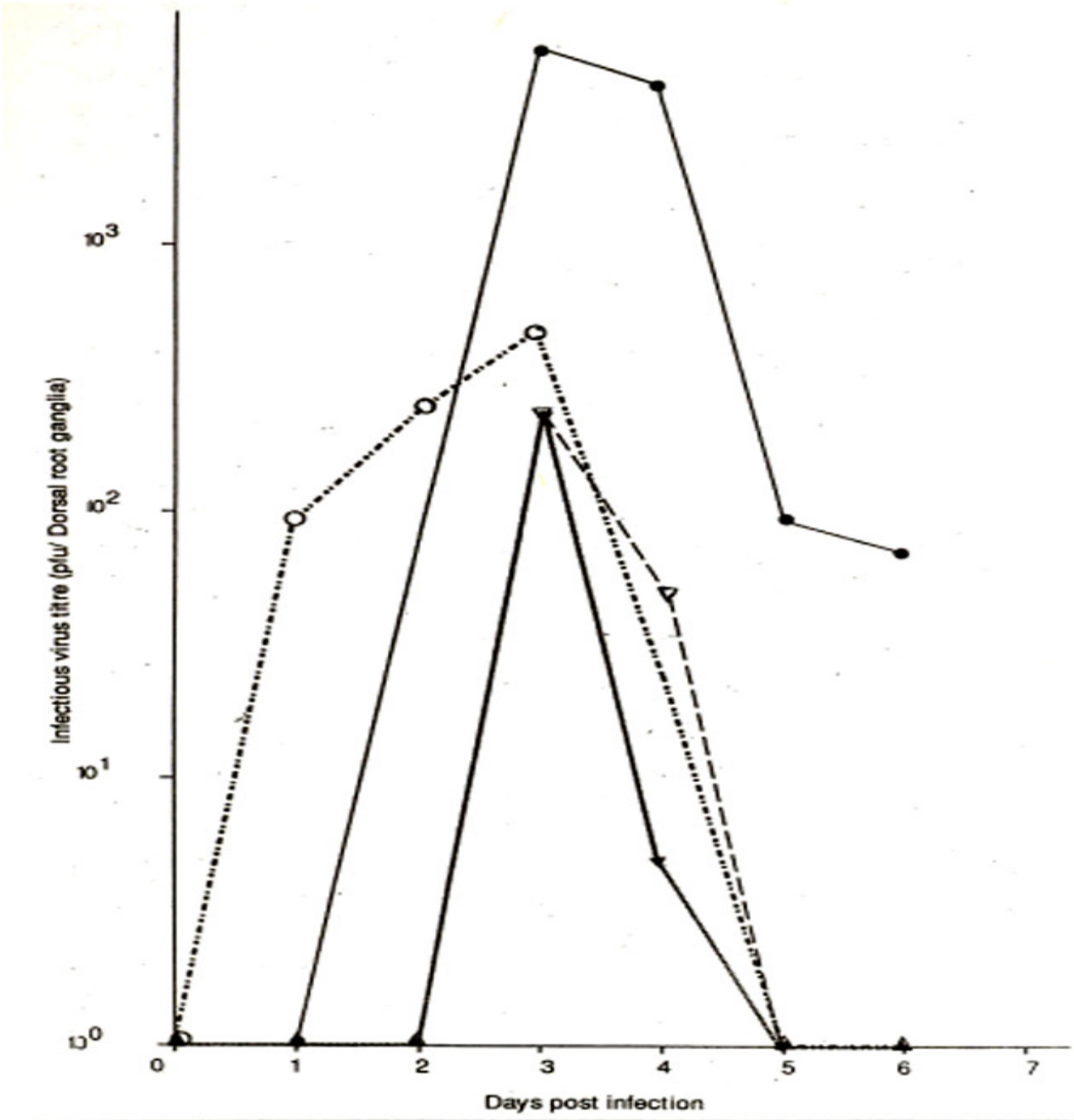

Figure 2. *In vivo* growth kinetics of HSV-I strain 17 syn+, 1704 and 1706. Three week old BALB/c mice were inoculated in the left rear foot pad with 25 ul of each virus with 17 syn+ (●), 1704 (▼), and 1706 (▽) at the input dose of $1x10^5$ pfu/mouse and 1704 (○) at the input dose of $1x10^7$ pfu/mouse. At indicated times post infection, two mice from each point were killed. The DRG from the left side of the spinal cord were removed and homogenized; the resulting cell suspension was sonicated and released infectious virus titrated on BHK-21 C13 cells at 37°C.

The results show (Figure 2) that the replication efficiency of 1704 and 1706 is poorwhen compared to wild type. At an input dose of 10^5 pfu/mouse 1704 and 1706 reached their peak on day three while on day five no virus was detectable. At an input dose of 107 pfu/mouse the variant 1704 was detected by day one post-inoculation and continued to increase in titre to the third day, but no vims was detectable by day five. In contrast growth of the wild type reached its peak on the third day post-inoculation, maintained its replication to day four and was detectable until day six. These results indicate that the growth of 1704 and 1706 is considerably impaired in the peripheral nervous system of the mouse,

which could explain the avimlence of 1704 and 1706 following foot -pad inoculation.

Discussion

Before studying pathogemcity of the variants in the mouse model system it was essential to determine the phenotype of the parental wild type virus. Therefore, we have evaluated the neurovirulence of individual plaque stocks isolated from the elite stock of 17 syn+ in order to determine the base line for evaluating deletion variants 1704, 1705 and 1706 and to further investigate any variation in pathogenicity among individual plaques in the elite stock of HS V-i strain 17 syn+. Although a proportion (upto 24%) of viable variants with divergent genomic structures in the population ofHSV-2 strain HG52 showed deletions in RL[12], the heterogeneity in the neurovimlence of individual plaque stocks of HSV-2 strain HG52 elite stock has been documented without detectable variation in the genome of the plaques inoculated intracrarnally in BALB/c mice[11]. The nine individual stocks isolated from the elite stocks of 17 syn+ showed no differences in their LD50 values following intracranial inoculation of three weeks old BALB/c mice. All the plaques have LD50\'s similar to the parental 17 syn+. Restriction endonuclease analysis of DNAs of the isolated plaques was carried out which showed no differences in the size and distribution of the fragments. The restriction endonuclease analysis however, cannot detect any point mutations, small deletions and/or insertions less than 150 bp in the genome, which could be a possible sources of variation in the pathogenic phenotype of the virus in mouse model. Intracranial inoculation of three week old BALB/c mice showed that the LD50 for 1705 was similar to the wild type virus but the difference with 1704 and 1706 compared to the wild type was approximately one log and two logs respectively i.e they were less virulent. Following foot pad inoculation 1705 behaved as the wild type but 1704 and 1706 were unable to kill any of the animals even at the highest possible infective dose of 1x107 pfu/mouse. The variants 1704 and 1706 failed to grow with the wild type kinetics and were undetectable by indicator cell tissue culture (BHK-21 C13) in the periphemi nervous system (DRG)by day four, can explain the inability of these viruses to kill any of the animal following foot-pad inoculation. The biological behaviour of 1704 could be due to —‘1 kb deletion involving the promoter region of latency associated transciipts (LATs) in temunal inverted repeat sequence of long region (TRL) and -4 kb deletion in internal inverted repeat sequence of long region/unique sequence of long region (IRL/UL) of the genome involving the entire LAT coding sequences and extending upto the UL56 gene sparing only 170 bp from its sequences[16] - The in vitro growth impairment of the 1706 has already been observed[13]. Although 1706 has similar deletion as 1705 (the deletion is -4.7kb between the 3 end of IEl and IE2 genes, removing IRL copy of LAT, UL55 and UL56 genes) except that it has sequences from nucleotide position (np) 9215 to np 13969 that have been repeated and inserted in an inverted orientation to replace the deletion. Consequently 1706 contains two copies of ULl, UL2, UL3 and UL4 gene alongwith a second partial copy ofUL5 gene resulting in the extended long repeats of the genome16. Possible explanation for this altered biological behaviour in 1706 could be (i) deletion of both the UL55 and UL56 genes[16], (ii) deletion of the sequences (YGTGTTY, where Y represents C orT) downstream from the 3 end of IE2 gene which play part in the effective tennination of mRNA from IE2 gene[16,19], (iii) double dose of all or one of the products ofthe genes ULI, UL2, UL3 and UL4[16]. These genes might be having an inhibitory effect on the gene regulatory system of the virus thus rendering its growth impaired. (iv) small deletions, insertions and/or point mutations elsewhere in the genome cannot be ruled out.

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References

1. Fenner, F.. Mcauslan, B.R., Mims, C.A. et a!. The Biology of animal viruses, 2nd edition, New York. Academic press, 1974, pp. 339-93.
2. Johnson, R.T. Viral infection of the nervous system. New York, Raven Press, 1982, pp. 129-56.
3. Caspery, L., Schilding, B.. Dundarov, S. et al. Infection ofsusceptible and resistant mouse strain with HSV-l and HSV-2. Arch. Virol., 1980; 65: 219-227.
4. Kohl, S. and Loo, L.S. Ontogeny ofmurine cellular cytotoxicity to herpes simplex virus infected cells. Infect. Immun., 1980; 30: 847-850.
5. Lopez, C. Genetics of natural resistance to herpesvirus infection in mice. Nature. 1975; 258: 152-153.
6. Dix, R.D., McKendall, R.R. and Baringer, JR. Comparative neurovirulence of herpes simplex virus type I strain after peripheral or intracerebral inoculation of BALB/c mice. Infect. Immun., 1983; 40:103-112.
7. Kaerner, H.C., Schroder, C.H., ott-Hartman, A. et at. Genetic variability of herpes simplex virus: Development of a pathogenic variant during passaging of a non-pathogenic herpes simplex virus type I strain in mouse brain. J. Virol., 1983;46,83-93.
8. Goodman, J.L. and Stevens. J.G. Passage of herpes simplex virus type I on chick embryo fibroblasts confers virulence for chick embryos. Virus Res., 1986;5:191-200.
9. Richards. J.T., Kern, E.R., Overall. J.C. Jr.. et at. Differences in neurovirulence among isolates of herpes simplex virus type I and type 2 using four routes of inoculation. J. Infect. Dis., 1981; 144: 1704-1715.
10. Javier, R.T., Sedarati, F. and Stevens, J.G. Two avirulent herpes simplex viruses generate lethal recombinant. Science, 1986; 234: 746-747.
11. Taha, MY., Brown, SM. and Clements G.B. Neurovirulence of individual plaque stocks of Herpes simplex virus 2 strain HG52. Arch. Virol., 1988; 103: 15-25.
12. Harland, J. and Brown, S.M. Isolation and characterization of deletion mutants of herpes simplex virus type 2 (strain HG52). J. Gen. Virol., 1985; 66: 1305-1321.
13. MacLean, R. J. and Brown, SM. Deletion and duplication variants around the long repeat region of herpes simplex virus type I strain 17. J. Gen. Virol., 1987;68:3021-31.
14. Chou, J., Kern, ER., Whitely, R.J. et al. Mapping of herpes simplex virus-1 neurovirulence to gamma! 34.5, a gene nonessential for growth in culture. Science, 1990; 250: 1262-1265.
15. Sussman, M.D., Lu, Z., Kutish, G., et al. Identification of an African swine fever virus gene with similarity to a myeloid differentiation gepe of herpes simplex virus. J. Virol., 1992; 66: 55 86-5589.
16. Junejo, F., MacLean, A.R. and Brown, SM. Sequence analysis of the herpes simplex virus type 1 strain 17 variants 1704, 1705 and 1706 with respect to their origin and effect on the latency associated transcript sequence. J. Gen. Virol., 1991;72:2311-2315.
17. Lonsdale, D.M. Arapid technique for distinguishing herpes simplex virus type 1 form type 2 by restriction technology. Lancet, 1979; 12: 849-852.
18. Reed, Li. and Muench, H. A simple method of estimating fifty percent endpoints. Amer. J. Hygiene, 1938; 27: 493-497.
19. Maclaughlan. J., Gaffney. D.. Whitton, J.L. et al. The consensus sequences YGTGTTY located downstream from the AATAAA signal is required for efficient formation of mRNA 3- termini. Nucleic. Acid. Res., 1985; 13: 1347-1368.