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Alphaherpesvirus DNA replication in dissociated human trigeminal ganglia

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Abstract

Analysis of the frequency and PCR-quantifiable abundance of herpes simplex virus type 1 (HSV-1) and varicella zoster virus (VZV) DNA in multiple biological replicates of cells from dissociated, randomly distributed human trigeminal ganglia (TG) of 4 subjects revealed an increase in both parameters and in both viruses during 5 days of culture, with no further change by 10 days. Dissociated TG provides a platform to analyze initiation of latent virus DNA replication within 5 days of culture.

Keywords

HSV-1; VZV; DNA; Human trigeminal ganglia; Reactivation

Introduction

HSV-1 and VZV are neurotropic alphaherpesviruses that become latent in human ganglia after primary infection. During latency, the respective 150-kbp and 125-kbp double-stranded DNA genomes are nonintegrated, histone-bound episomes from which virus transcription is limited to a restricted set of genes (Eshleman et al. 2011). Virus reactivation results in disease typically more serious than after primary infection. HSV-1 reactivation produces recurrent oral lesions, blindness due to herpes stromal keratitis and rarely fatal encephalitis (Mitchell et al. 2003). VZV reactivation most often results in zoster (shingles), frequently followed by chronic pain (postherpetic neuralgia) (Gilden et al. 2003). Zoster is also complicated by paralysis and incontinence (VZV myelopathy), headache and cognitive impairment (VZV meningoencephalitis), blindness (VZV retinitis), and stroke (VZV vasculopathy) (Gilden et al. 2010; Nagel et al. 2012). Recently, VZV has been recognized as the trigger for the immunopathology of giant cell arteritis (Gilden et al., 2015; Nagel et al. 2015).

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Conflict of interest

All authors report no conflict of interest.

The ability to model HSV-1 latency in mice (Morahan et al. 1979) and rabbits (Shimomura et al. 1983) has greatly advanced the understanding of virus reactivation (Webre et al. 2012). However, VZV is a uniquely human virus and the lack of an animal model has hindered insight into its reactivation (Shahzad et al. 2015). Thus, we focused our present analyses on adult human trigeminal ganglia (TG), where both HSV-1 and VZV are latent (Cohrs et al. 2005). Previously, both HSV-1 and VZV DNA replication was observed in human TGs cut into equal pieces and cultured for 0, 1 and 5 days (Azarkh et al. 2012). However, latent HSV-1 and VZV DNA are not uniformly distributed in human TG (Cohrs et al. 2005; Hübner et al. 2009) and a more accurate analysis of virus DNA replication in human TG requires confirmation that dissociated ganglia are randomly distributed among multiple samples. Our current study using finely dissociated human TG divided into 21 aliquots enabled statistical analysis to ensure sample homogeneity. Assays of neuronal viability and virus DNA at 0, 5 and 10 days in left and right human TGs indicated that: neurons remained viable throughout the study; TG dissociation yielded multiple, randomly distributed biological samples; and both VZV and HSV-1 DNA increased from 0 to 5 days and remained constant thereafter. While no drug was added to the cultures to inhibit virus DNA replication, our data strongly suggests that the events that initiate latent virus DNA replication in human TG occur within 5 days post-explantation.

In accordance with approved protocols (Colorado Multiple Institutional Review Board) and OHRP (<http://www.hhs.gov/ohrp>) and FDA (<http://www.fda.gov/oc/ohrt/irbs/default.htm>) guidelines and with informed consent from next-of-kin, human right and left TG were removed from 4 individuals with no cutaneous signs of herpesvirus infection and who were not immunocompromised before death (Table 1). TG were washed and mechanically dissociated. Cells were collected (1000 g, 10 min, 4°C), resuspended in 3 ml of complete culture medium #2 lacking anti-mitotic drugs (Azarkh et al. 2012) and distributed into 7 wells in each of three 96-well poly-L-ornithine/laminin-coated plates (BD-Biocoat, Fisher Biosciences, Vacaville, CA). Cells were not sorted before use and contained neurons, satellite cells, which enhance neuronal survival (Hanai et al. 2011), and other non-neuronal cells. Samples were harvested after 0, 5 and 10 days of incubation at 37°C by mechanically detaching cells from the plate into the tissue culture medium and collecting all cells by low speed centrifugation. Neurons were readily identified by large size, characteristic cytoplasmic lipofuscin inclusions (Fig. 1, left panel, black arrows) and frequent axon-like extensions (left panel, yellow arrows). The small satellite cells surrounding neurons were difficult to visualize with tungsten light (left panel), but were readily identified by DAPI staining (Fig. 1, middle panel). Uptake of red fluorescent vital dye (Cell Tracker; Invitrogen, Grand Island, NY) indicated the viability of the neuron-satellite cell complexes at 0 and 10 days of incubation (Fig. 1, middle and right panels, respectively).

Results and discussion

DNA was extracted from each culture and PCR-amplified using TaqMan primers specific for VZV, HSV-1 and GAPdH (Nagel et al. 2011). While each PCR contained dilutions of HSV-1 and VZV DNA permitting quantitation of virus genome copies, PCR results are given as cycle threshold (Ct) values (Table 2), since multiple PCRs using known copies of virus DNA showed that Ct values >35 indicated the presence of virus DNA, but at levels too low for

quantitative analysis. At day 0, GAPdH Ct values for the 7 biological replicates from each TG reflected random aliquots from each TG as shown by Kruskal-Wallis test ($p=0.1$ to control for type 1 false-positive error). GAPdH sequences were amplified from all 112 PCRs (7 biological replicates per TG, 2 TGs from each of 4 subjects, in duplicate). HSV-1 DNA was detected in at least 1 of 7 biological replicates from 7 of 8 TGs, in 73 of 112 (65%) PCRs ($Ct<40$) and was quantifiable in 49 of 112 (44%) PCRs ($Ct<35$). Similarly, VZV DNA was detected in at least 1 of 7 biological replicates from all 8 TGs, in 46 of 112 (41%) PCRs ($Ct<40$) and was quantifiable in 11 of 112 (10%) PCRs.

At day 5, GAPdH sequences were quantifiable ($Ct<35$) in all 112 PCRs. HSV-1 DNA was detected ($Ct<40$) in at least one biological replicate from 7 of 8 TGs, in 74 of 112 (64%) PCRs and was quantifiable in 70 of 112 (62%) PCRs. Similarly, VZV DNA was detected in at least 1 of 7 biological replicates from each TG, in 88 of 112 (78%) PCRs ($Ct<40$) and was quantifiable in 43 of 112 (38%) PCRs ($Ct<35$).

At day 10, GAPdH DNA was quantifiable in all 112 PCRs. HSV-1 DNA was detected ($Ct<40$) in at least one biological replicate from 6 of 8 TGs, in 76 of 112 (67%) PCRs and was quantifiable in 54 of 112 (48%) PCRs. Similarly, VZV DNA was detected in at least 1 of 7 biological replicates from each TG, in 88 of 112 (78%) PCRs ($Ct<40$) and was quantifiable in 51 of 112 (45%) PCRs ($Ct<35$).

Our data that showed HSV-1 DNA in 3 of 4 (75%) and VZV DNA in 4 of 4 (100%) individuals compared favorably with previous findings showing HSV-1 DNA in TG from 8 of 11 (73%) and VZV DNA in TG from 10 of 11 (93%) individuals (Mahalingam et al. 1992), HSV-1 in TG from 8 of 15 (15%) individuals and VZV in TG from 13 of 15 (87%) individuals (Pevenstein et al. 1999), and HSV-1 in TG from 12 of 17 (70%) individuals and VZV in TG from 17 of 17 (100%) individuals (Cohrs et al. 2000). Also, on average, we found more copies of HSV-1 DNA copies than copies of VZV DNA, data consistent with Pevenstein et al. (1999).

General patterns of virus DNA replication become apparent upon analysis of the rate of virus DNA increase for each TG as a function of incubation time (fold DNA increase = $2^{-(Ct_{X1}-Ct_{X0})}$, where $(X_0, X_1) = (\text{day 0, day 5})$ or (day 5, day 10) (Fig. 2). After 5 days in culture, HSV-1 DNA increased more than 2-fold in 4 of 6 TGs, while VZV DNA increased more than 2-fold in 2 of 5 TGs. By 10 days, the rate of DNA accumulation for both viruses decreased significantly, most likely indicating that the major events initiating latent virus DNA replication occur within 5 days in culture. An alternative explanation is that virus DNA replication extended through the region detected by qPCR, but did not proceed to completion. To test this hypothesis would require deep-sequencing virus DNA in the samples which is beyond the scope of the current report.

The TGs from subject 4 did not contain HSV-1 DNA, while the TGs from subjects 1–3 contained both HSV-1 and VZV DNA, permitting analysis of virus DNA copy numbers when one or both viruses were present in dissociated TG samples. The increase in VZV DNA at day 5 in ganglia with only latent VZV DNA (subject 4) was greater than in ganglia containing both HSV-1 and VZV DNA (subjects 1–3), raising the possibility that the

presence of latent HSV-1 inhibits VZV DNA replication. Further analyses of additional human TG are needed to test these possibilities.

HSV-1 and VZV can be found in the same human neuron (Theil et al. 2003), although the frequency of dual infection is unknown. Our data indicate the simultaneous presence of HSV-1 and VZV in human ganglia, as well as their concurrent DNA replication. HSV-1 reactivation was detected in human TG explants from 1 of 22 subjects after 3 weeks of co-cultivation on permissive Vero cells (Bastian et al. 1972) and in 21 of 53 (40%) cultures from 6 subjects after 10 to 45 days in culture (Baringer and Swoveland 1973); however, the use of similar protocols revealed no VZV reactivation in approximately 100 thoracic ganglia (4–6 ganglia from each of 20 adult cadavers) (Plotkin et al. 1977). We did not assess production of infectious virus since our goal here was to analyze alphaherpesvirus DNA replication after TG dissociation.

Our results show that dissociated adult human TG surrounded by supporting satellite cells in a milieu of associated non-neuronal cells remain viable for 10 days and that at day 5, both the number of virus-positive samples and the virus DNA content increases significantly. While further analysis of the 5-day interval, including assays for release of infectious virus, is needed, our assays in which dissociated adult neurons remain viable in culture provide a platform to study early molecular events leading to HSV-1 and VZV DNA replication.

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Fig. 1.

Dissociation of human trigeminal ganglia. A human trigeminal ganglion was dissociated and cultured for 0 (left and middle panels) or 10 (right panel) days. Lipofuscin (left panel, black arrows) and axon-like processes (yellow arrows) characteristic of neurons are shown. Satellite cells (middle panel, white arrows) surround neurons intensely stained with red fluorescent cell tracker, an indicator of viable cells. At day 10, neurons surrounded by blue (DAPI)-staining satellite cells were still viable (red dye in right panel).

Fig. 2.

PCR analysis of HSV-1 and VZV DNA from human trigeminal ganglia. DNA extracted from each of 21 cultures of dissociated human trigeminal ganglia was quantitated by PCR and fold DNA increase was calculated with respect to virus DNA levels present at day 0.

Table 1

Clinical features of humans from whom trigeminal ganglia was removed

Subject	Age (yr)	Gender	Cause of death	Time (h) from death to autopsy
1	18	M	Auto accident	24
2	60	M	Overdose, cocaine	14
3	56	F	Natural, lupus complications	18
4	27	M	Natural, Morquio's syndrome complications	14

Table 2

Human trigeminal ganglia amplified with primers for HSV-1, VZV and cell (GAPDH)

ID	Sample	Day 0				Day 5				Day 10			
		HSV-1 ^a	VZV ^b	GAPDH ^c	HSV-1 ^a	VZV ^b	GAPDH ^c	HSV-1 ^a	VZV ^b	GAPDH ^c	HSV-1 ^a	VZV ^b	GAPDH ^c
1	LTG ^d	UD ^e /UD	UD/UD	25.93/26.00	UD/NQ ^f	NQ/UD	24.52/24.76	UD/UD	34.27/34.57	24.24/24.8	UD/UD	NQ/NQ	25.19/25.94
	LTG	UD/UD	UD/UD	26.46/26.44	UD/UD	NQ/UD	25.72/25.9	UD/UD	UD/UD	26.88/27.12	NQ/NQ	UD/UD	26.55/25.45
	LTG	NQ/NQ	UD/UD	27.67/27.68	UD/UD	UD/UD	26.23/26.12	NQ/UD	34.74/NQ	25.15/25.17	UD/UD	NQ/NQ	24.69/25.04
	LTG	UD/UD	UD/UD	26.97/26.63	NQ/UD	UD/UD	26.44/26.27	NQ/NQ	NQ/NQ	25.02/24.82	NQ/NQ	NQ/NQ	25.43
	LTG	UD/UD	UD/UD	28.05/27.58	UD/UD	UD/UD	26.23/26.04	UD/UD	0.87	0.87	UD/UD	NQ/NQ	0.87
	LTG	UD/NQ	UD/UD	28.26/27.94	UD/UD	UD/UD	26.10/26.05	UD/UD	0	0	UD/UD	NQ/NQ	0
	LTG	UD/UD	NQ/NQ	28.07/27.09	UD/NQ	UD/UD	26.36/26.05	NQ/NQ	3	3	UD/UD	NQ/NQ	3
	ave Ct ^g	NA ^h	NA	27.20	NA	NA	25.91	NA	6	6	NA	NA	6
	std Ct ⁱ	NA	NA	0.80	NA	NA	0.57	NA	14	14	NA	NA	14
	N (Ct 35) ^j	0	0	14	0	0	14	0	2	2	0	0	2
	N (Ct <40) ^k	3	2	14	3	2	14	6	14	14	6	12	14
	N (total) ^l	14	14	14	14	14	14	14	14	14	14	14	14
	RTG	32.38/32.37	UD/UD	22.05/22.20	32.00/32.03	UD/UD	23.49/23.45	32.42/32.31	NQ/NQ	23.48/23.77	32.42/32.31	NQ/NQ	23.86/23.85
	RTG	UD/UD	UD/UD	24.83/24.64	31.53/31.82	UD/UD	23.17/23.02	33.31/33.32	NQ/NQ	23.86/23.85	33.31/33.32	NQ/NQ	23.91/23.58
	RTG	NQ/NQ	UD/UD	25.63/25.44	32.24/33.53	UD/NQ	23.40/23.26	32.83/33.13	NQ/NQ	23.91/23.58	32.83/33.13	NQ/NQ	23.64/23.56
	RTG	NQ/NQ	UD/NQ	25.17/25.24	33.17/33.13	UD/NQ	23.26/23.33	33.27/33.18	NQ/NQ	23.64/23.56	33.27/33.18	NQ/NQ	23.28/23.26
	RTG	NQ/NQ	UD/NQ	25.32/25.16	32.90/33.34	NQ/NQ	23.59/23.39	34.00/33.62	NQ/NQ	23.21/23.29	34.00/33.62	NQ/NQ	23.17/23.2
	RTG	NQ/NQ	UD/UD	26.20/25.91	31.96/33.09	UD/UD	23.60/23.44	33.55/33.39	NQ/UD	23.21/23.29	33.55/33.39	NQ/UD	23.17/23.2
	RTG	33.85/34.28	UD/UD	25.43/25.54	33.00/32.67	UD/NQ	23.73/23.89	33.37/33.36	NQ/NQ	23.17/23.2	33.37/33.36	NQ/NQ	23.17/23.2
	ave Ct	33.66	NA	24.91	32.60	NA	23.43	33.22	NA	23.50	33.22	NA	23.50
	std Ct	1.31	NA	1.24	0.65	NA	0.23	0.45	NA	0.27	0.45	NA	0.27
	N (Ct 35)	5	0	14	14	0	14	14	0	14	14	0	14
	N (Ct <40)	12	2	14	14	5	14	14	13	14	14	13	14
	N (total)	14	14	14	14	14	14	14	14	14	14	14	14

ID	Sample	Day 0			Day 5			Day 10		
		HSV-1 ^a	VZV ^b	GAPuH ^c	HSV-1 ^a	VZV ^b	GAPuH ^c	HSV-1 ^a	VZV ^b	GAPuH ^c
	water	UD/UD	UD/NQ	UD/NQ	UD/UD	NQ/UD	UD/UD	UD/UD	UD/UD	UD/UD
	water	UD/UD	UD/UD	UD/UD	UD/UD	UD/UD	UD/UD	UD/UD	UD/UD	NQ/UD
					UD/UD	UD/UD	UD/UD	UD/UD	UD/UD	UD/UD
					UD/UD	UD/UD	UD/UD	UD/UD	NQ/UD	UD/NQ
	ave Ct	NA	NA	NA	NA	NA	NA	NA	NA	NA
	std Ct	NA	NA	NA	NA	NA	NA	NA	NA	NA
	N _(Ct<35)	0	0	0	0	0	0	0	0	0
	N _(Ct<40)	0	1	1	0	1	0	0	1	1
	N _(total)	4	4	4	8	8	8	8	8	8
2	LTG	33.35/34.32	UD/UD	24.83/24.50	30.93/30.89	35.00/34.15	23.04/23.04	29.12/28.96	34.22/34.02	22.67/22.55
	LTG	35.00/35.00	NQ/UD	26.56/26.25	30.4/30.73	NQ/34.81	24.14/24.08	27.87/27.65	33.98/33.35	22.65/22.59
	LTG	32.55/32.35	UD/UD	24.86/24.88	30.69/30.46	34.57/34.47	23.88/23.79	28.78/28.83	34.81/34.01	23.00/23.07
	LTG	34.82/34.32	UD/UD	25.36/25.52	31.21/31.12	33.48/34.25	24.15/24.03	29.32/29.54	NQ/NQ	23.12/23.29
	LTG	35.23/NQ	UD/NQ	25.76/26.00	30.59/30.73	NQ/NQ	24.41/24.16	30.26/30.45	NQ/34.79	23.04/23.12
	LTG	34.05/33.73	NQ/NQ	25.82/25.93	30.97/31.14	34.95/34.55	24.58/24.22	30.38/30.61	NQ/34.92	23.32/23.51
	LTG	32.43/32.37	UD/UD	25.33/25.28	30.70/31.37	NQ/NQ	24.73/24.29	29.72/30.08	34.32/33.64	23.18/23.23
	ave Ct	33.81	NA	25.49	30.85	34.47	24.04	29.40	34.21	23.02
	std Ct	1.09	NA	0.60	0.29	0.47	0.49	0.93	0.52	0.30
	N _(Ct<35)	13	0	14	14	9	14	14	10	14
	N _(Ct<40)	14	4	14	14	14	14	14	14	14
	N _(total)	14	14	14	14	14	14	14	14	14
	RTG	NQ/NQ	UD/NQ	24.88/24.62	31.09/31.62	NQ/NQ	24.28/24.36	29.94/30.41	33.40/34.27	22.66/22.91
	RTG	34.53/NQ	NQ/NQ	26.52/26.29	31.08/30.98	35.16/35.28	24.15/24.20	31.45/32.10	35.23/35.10	23.68/23.68
	RTG	34.69/34.81	NQ/NQ	24.85/24.66	31.29/31.48	34.52/34.23	24.33/24.28	29.29/29.61	34.92/34.53	23.37/23.38
	RTG	NQ/34.49	UD/NQ	25.18/24.97	30.5/30.67	33.82/34.67	23.98/23.76	29.98/30.11	33.07/32.87	23.21/23.22
	RTG	NQ/34.94	UD/UD	25.41/25.29	31.39/31.66	34.02/33.68	23.99/23.76	30.68/31.08	34.26/35.22	23.37/23.50
	RTG	34.72/34.85	NQ/NQ	25.25/25.13	31.92/32.14	NQ/NQ	23.91/23.72	31.11/31.39	34.25/34.35	23.21/23.37

ID	Sample	Day 0			Day 5			Day 10		
		HSV-1 ^a	VZV ^b	GAPdH ^c	HSV-1 ^a	VZV ^b	GAPdH ^c	HSV-1 ^a	VZV ^b	GAPdH ^c
RTG		NQ/NQ	NQ/UD	24.82/24.66	32.03/32.22	NQ/NQ	24.14/23.96	31.08/31.13	34.78/35.74	23.70/23.80
ave Ct		34.72	NA	25.18	31.43	34.16	24.06	30.67	34.07	23.36
std Ct		0.16	NA	0.58	0.54	0.39	0.22	0.80	0.71	0.32
N (Ct 35)		7	0	14	14	6.00	14	14	10	14
N (Ct<40)		14	9	14	14	14.00	14	14	14	14
N (total)		14	14	14	14	14.00	14	14	14	14
water		UD/UD	UD/UD	NQ/UD	UD/UD	UD/UD	UD/UD	UD/UD	UD/UD	UD/UD
water		UD/UD	UD/UD	UD/UD	UD/UD	UD/UD	UD/UD	UD/UD	UD/UD	UD/UD
					UD/UD	UD/UD	UD/UD	UD/UD	UD/UD	UD/UD
					UD/UD	UD/UD	NQ	UD/UD	UD/UD	UD/UD
ave Ct		NA	NA	NA	NA	NA	NA	NA	NA	NA
std Ct		NA	NA	NA	NA	NA	NA	NA	NA	NA
N (Ct 35)		0	0	0	0	0	0	0	0	0
N (Ct<40)		0	0	1	0	0	1	0	0	0
N (total)		4	4	4	8	8	8	8	8	8
3	LTG	33.86/33.74	34.87/NQ	24.82/24.95	30.17/30.45	NQ/NQ	22.51/22.60	34.17/34.49	UD/UD	23.07/23.17
	LTG	NQ/34.53	NQ/UD	24.96/24.99	30.55/30.61	UD/NQ	23.01/23.12	34.29/34.24	UD/UD	22.57/22.70
	LTG	NQ/33.43	UD/UD	24.96/24.92	30.26/30.14	NQ/NQ	23.07/23.24	NQ/NQ	UD/UD	23.44/23.76
	LTG	NQ/NQ	NQ/UD	25.81/25.94	31.47/31.66	NQ/NQ	23.75/24.10	NQ/NQ	UD/UD	23.97/24.20
	LTG	NQ/33.38	UD/UD	25.52/25.36	31.03/31.14	NQ/NQ	23.36/23.98	NQ/34.81	UD/UD	23.35/23.52
	LTG	NQ/36.00	UD/UD	26.23/26.38	31.21/31.02	NQ/NQ	23.64/23.70	NQ/NQ	UD/NQ	24.49/24.59
	LTG	34.68/33.63	NQ/UD	25.49/25.45	31.54/31.39	NQ/NQ	23.70/23.71	NQ/NQ	UD/UD	24.64/24.55
ave Ct		34.13	34.87	25.41	30.90	NA	23.39	34.40	NA	23.72
std Ct		0.83	NA	0.52	0.53	NA	0.49	0.26	NA	0.71
N (Ct 35)		8	1	14	14	0	14	5	0	14
N (Ct<40)		14	5	14	14	13	14	14	1	14

ID	Sample	Day 0			Day 5			Day 10			
		HSV-1 ^a	VZV ^b	GAPdH ^c	HSV-1 ^a	VZV ^b	GAPdH ^c	HSV-1 ^a	VZV ^b	GAPdH ^c	
		14	14	14	14	14	14	14	14	14	
4	N _(total)		14	14	14	14	14	14	14	14	14
	RTG	30.86/31.05	NQ/UD	22.87/22.90	28.61/28.33	NQ/34.64	22.35/22.44	32.25/31.74	35.02/36.51	21.79/21.74	
	RTG	33.55/33.49	UD/UD	24.79/24.81	29.05/29.04	NQ/NQ	22.73/22.76	34.10/33.63	36.74/37.6	23.95/23.99	
	RTG	34.76/33.93	NQ/UD	24.58/24.63	29.39/29.65	NQ/NQ	22.51/22.54	34.05/33.57	40/36.21	24.51/24.67	
	RTG	33.09/32.84	UD/UD	25.07/25.11	29.95/29.68	34.00/33.84	23.00/23.06	NQ/34.91	37.93/40	25.25/25.6	
	RTG	33.03/32.41	UD/UD	24.11/24.20	30.62/30.80	NQ/NQ	23.05/23.02	NQ/NQ	40/40	25.23/25.34	
	RTG	33.12/33.06	NQ/NQ	24.54/24.76	33.51/33.15	NQ/NQ	24.52/24.70	NQ/NQ	40/40	25.07/25.28	
	RTG	34.59/33.72	UD/UD	24.88/25.00	31.32/31.11	NQ/NQ	23.57/23.70	NQ/NQ	40/40	25.13/25.42	
	ave Ct	33.11	NA	24.45	30.30	34.16	23.14	33.46	NA	24.50	
	std Ct	1.12	NA	0.72	1.57	0.42	0.74	1.10	NA	1.26	
	N _(Ct 35)	14	0	14	14	3	14	7	0	14	
	N _(Ct<40)	14	4	14	14	14	14	14	6	14	
N _(total)	14	14	14	14	14	14	14	14	14		
4	water	UD/UD	UD/NQ	UD/NQ	UD/UD	NQ/UD	UD/UD	UD/UD	UD/UD	UD/UD	
	water	UD/UD	UD/UD	UD/UD	UD/UD	UD/UD	UD/UD	UD/UD	UD/UD	UD/UD	
	ave Ct	NA	NA	NA	NA	NA	NA	NA	NA	NA	
	std Ct	NA	NA	NA	NA	NA	NA	NA	NA	NA	
	N _(Ct 35)	0	0	0	0	0	0	0	0	0	
	N _(Ct<40)	0	1	1	0	0	0	0	1	1	
	N _(total)	4	4	4	8	8	8	8	8	8	
	LTG	UD/UD	UD/UD	23.91/23.86	UD/UD	30.68/30.73	19.69/20.04	UD/UD	31.14/31.3	21.70/21.90	
	LTG	33.33/33.17	NQ/NQ	23.07/23.11	UD/UD	32.22/32.29	21.04/21.15	UD/UD	30.05/30.41	21.01/21.06	
	LTG	UD/UD	35.44/35.00	22.99/23.07	UD/UD	32.55/32.07	22.00/22.12	UD/UD	31.22/31.77	21.27/21.34	
	LTG	UD/UD	32.63/34.67	22.68/23.07	UD/UD	32.32/32.46	22.05/22.15	UD/UD	31.29/31.33	21.74/21.98	
	LTG	UD/UD	33.25/33.62	22.52/22.67	UD/UD	31.82/32.13	21.57/21.67	UD/UD	31.19/31.14	21.52/21.72	

ID	Sample	Day 0			Day 5			Day 10		
		HSV-1 ^a	VZV ^b	GAPdH ^c	HSV-1 ^a	VZV ^b	GAPdH ^c	HSV-1 ^a	VZV ^b	GAPdH ^c
LTG		UD/UD	34.14/34.4	23.77/24.00	UD/UD	32.25/32.05	21.59/21.75	UD/UD	31.01/31.02	21.79/22.05
LTG		UD/UD	33.26/34.81	23.71/23.67	UD/UD	33.27/33.79	23.07/22.94	UD/UD	31.42/32.04	22.48/22.30
ave Ct		33.25	33.98	23.29	#DIV/0!	32.19	21.63	NA	31.17	21.70
std Ct		0.12	0.82	0.51	#DIV/0!	0.81	0.94	NA	0.49	0.43
N (Ct 35)		2	9	14	0.00	14	14	0	14	14
N (Ct<40)		2	12	14	0.00	14	14	0	14	14
N (total)		14	14	14	14.00	14	14	14	14	14
RTG		UD/UD	40/40	24.81/24.63	UD/38.22	30.61/31.88	20.26/21.10	UD/UD	30.27/30.56	20.14/20.24
RTG		UD/UD	40/40	23.65/23.51	UD/UD	31.16/31.72	20.61/21.29	UD/UD	30.23/30.55	20.87/21.05
RTG		UD/UD	40/38.59	23.61/23.48	UD/UD	31.14/31.77	21.05/21.60	UD/UD	30.92/31.03	21.03/21.16
RTG		UD/UD	40/37.5	24.52/24.2	UD/UD	31.70/31.72	20.44/20.98	UD/UD	30.91/30.98	20.88/20.8
RTG		UD/UD	35.85/38.44	25.09/24.98	UD/UD	31.85/32.09	20.83/21.37	UD/UD	30.28/30.41	21.03/21.02
RTG		UD/UD	38.83/38.79	23.89/23.66	UD/UD	32.18/32.11	21.47/22.02	UD/UD	30.77/31.56	20.70/20.96
RTG		UD/UD	34.84/35.85	24.23/24.25	UD/UD	32.87/32.97	22.24/22.83	UD/UD	30.52/30.92	21.39/21.54
ave Ct		NA	34.84	24.18	NA	32.01	21.29	NA	30.71	20.92
std Ct		NA	NA	0.56	NA	0.53	0.71	NA	0.37	0.38
N (Ct 35)		0	1	14	0	11	14	0	14	14
N (Ct<40)		0	8	14	1	12	14	0	14	14
N (total)		14	14	14	14	14	14	14	14	14
water		UD/UD	UD/UD	NQ/UD	UD/UD	UD/UD	NQ/UD	UD/UD	UD/UD	UD/UD
water		UD/UD	UD/UD	UD/UD	UD/UD	UD/UD	UD/UD	UD/UD	UD/UD	UD/UD
					UD/UD	UD/UD	UD/UD	UD/UD	UD/UD	UD/UD
					UD/UD	UD/UD	UD/UD	UD/UD	UD/UD	UD/NQ
ave Ct		NA	NA	NA	NA	NA	NA	NA	NA	NA
std Ct		NA	NA	NA	NA	NA	NA	NA	NA	NA
N (Ct 35)		0	0	0	0	0	0	0	0	0

ID	Sample	Day 0			Day 5			Day 10		
		HSV-1 ^a	VZV ^b	GAPdH ^c	HSV-1 ^a	VZV ^b	GAPdH ^c	HSV-1 ^a	VZV ^b	GAPdH ^c
	N _(Ct<40)	0	0	1	0	0	1	0	0	1
	N _(total)	4	4	4	8	8	8	8	8	8

^a HSV-1 DNA PCR results for replicated samples (sample 1/sample 2)

^b VZV DNA PCR results for replicated samples (sample 1/sample 2)

^c GAPdH DNA PCR results for replicated samples (sample 1/sample 2)

^d LTG, left trigeminal ganglion

^e UD, undetected (Ct > 40)

^f NQ, not quantifiable (35 < Ct < 40)

^g ave Ct, average cycle threshold

^h NA, not applicable

ⁱ sid Ct, standard deviation of the cycle threshold

^j N(Ct<V40), number of PCRs with cycle threshold <40

^k N(Ct<35), number of PCRs with cycle threshold <35

^l N(total), total number of PCR assays with cycle threshold <35