Journal of NeuroVirology, 10: 216–222, 2004

ISSN: 1355-0284 print / 1538-2443 online
DOI: 10.1080/13550280490463569

Taylor & Francis
Taylor & Francis Group

Quantitative analysis of herpes simplex virus in cranial nerve ganglia

Jeffrey T Vrabec and Raye L Alford

Department of Otorhinolaryngology and Communicative Studies, Baylor College of Medicine, Houston, Texas, USA

A susceptible individual exposed to herpes simplex virus (HSV) will develop latent infection in multiple cranial nerve ganglia. There are a few quantitative studies of the viral load within the trigeminal ganglion, but none that investigate other cranial nerve ganglia. In this study, human trigeminal, geniculate, vestibular (Scarpa's) and cochlear (spiral) ganglia were obtained from willed body donors. Real time quantitative polymerase chain reaction (PCR) analysis of the HSV DNA polymerase gene was performed on ipsilateral ganglion sets from the same individual. Viral load, expressed as HSV genomes per 10^5 cells, was significantly greater in the vestibular ganglion (mean $\pm$ SD, 176705 $\pm$ 255916) than in the geniculate (9948 $\pm$ 22066), cochlear (3527 $\pm$ 9360), or trigeminal (2017 $\pm$ 5578) ganglia. There was not a significant correlation among ganglia from the same individual. The results support the hypothesis that neuronal subpopulations have variable susceptibility to HSV infection. *Journal of NeuroVirology* (2004) **10,** 216–222.

Keywords: cranial nerve ganglion; herpes simplex virus; quantitative PCR; viral load

Introduction

The study of herpes simplex virus (HSV) in cranial nerve sensory ganglia initially focused on detection and prevalence studies (Bastian *et al*, 1972; Baringer and Swoveland 1973; Warren *et al*, 1978). As the sensitivity of detection methods improves, prevalence figures are revised, leading to the conclusion that latent infection of cranial nerve ganglia with HSV is a common event. Contemporary autopsy studies in adult subjects define the prevalence of HSV in the trigeminal ganglion to be approximately 70% (Mahalingham *et al*, 1992; Liedtke *et al*, 1993; Pevenstein *et al*, 1999; Cohrs *et al*, 2000).

The trigeminal ganglion has been the focus of most investigations for several reasons. First, it is easily accessible. Second, the clinical features of HSV reactivation in the sensory distribution of the ganglion are well recognized. Examples include herpetic keratitis and herpes labialis. Finally, reliable, reproducible animal models have been developed that mimic human ocular herpes providing additional confirmation of the viral etiology (Carr *et al*, 2001).

Other cranial nerve ganglia have received less attention. Prevalence figures for HSV in the geniculate, cochlear, vestibular, and vagal ganglia are similar to those seen in the trigeminal ganglion (Furuta *et al*, 1992; Schulz *et al*, 1998; Vrabec and Payne, 2001). Although the clinical relevance of these findings remains to be determined, viral infection of a ganglion would be a prerequisite for any clinical syndrome purported to result from HSV reactivation. More definitive evidence of viral reactivation is obtained when replicating virus is isolated from the ganglion of interest in concert with the clinical symptoms. For example, isolation of HSV from the geniculate ganglion during acute facial paralysis has strengthened the association of HSV reactivation and Bell's palsy (Murakami *et al*, 1996).

Unfortunately, dissection or biopsy of most cranial nerve ganglia during one's lifetime is impractical. Thus, autopsy data revealing HSV DNA in the vestibular and cochlear ganglia have intensified speculation that the virus is responsible for various otologic clinical syndromes such as vestibular neuronitis and Meniere's disease (Gacek and Gacek, 2002; Vrabec, 2003). In the same manner, HSV reactivation

Address correspondence to Jeffrey T. Vrabec, MD, Baylor College of Medicine, Department of Otolaryngology and Communicative Studies, 6550 Fannin, Suite 1727, Houston, TX 77030, USA. E-mail: jvrabec@bcm. tmc.edu

Received 24 September 2003; revised 29 December 2003; accepted 16 February 2004.

from the vagal ganglion is suspected in various laryngeal and gastroesophageal disorders, including gastroesophageal reflux, herpetic laryngitis, and gastric ulcers (Gesser *et al*, 1995; Vrabec *et al*, 2000; Amin and Koufman, 2001).

The neuron is the reservoir for latent HSV infection in animal models and human ganglion sections. Initial reports were based on *in situ* hybidization techniques (Croen *et al*, 1987; Tenser *et al*, 1982). Subsequent confirmation was achieved using various methods of cell separation, including dissociation and fractionation according to size and morphology, or laser capture microdissection (Sawtell, 1997; Chen *et al*, 2002; Cai *et al*, 2002). These methods also confirm the absence of HSV DNA in supporting cells.

Current data suggest infection of all neurons within a ganglion does not occur and that the number of viral genome copies per cell is not uniform. The percentage of infected neurons and the number of viral copies per cell directly correlate with efficiency of reactivation in animal models (Maggioncalda *et al*, 1996; Sawtell, 1998; Sawtell *et al*, 1998). Factors important in determining viral load include infecting inoculum titer, virus strain, and viral replication within the ganglion (Sawtell, 1998; Sawtell *et al*, 1998; Thompson and Sawtell, 2000). To date, these observations have not been verified in a human population; however, one could postulate that individuals with more frequent clinical symptoms of viral reactivation harbor a greater viral load in their ganglia. Therefore, considerable interest exists in defining percentage of infected neurons and viral genome copies per cell in human ganglia.

Results

Twelve sets of HSV positive ganglia were evaluated by quantitative analysis. The data are summarized in Table 1. The mean HSV genome copy number per 10^5 cells in the vestibular ganglion was significantly greater than in the other ganglia (analysis of variance [ANOVA], $P = .01$). There was no significant difference in viral load among the other ganglia. The median HSV genome copy number per 10^5 cells (and range) for each ganglion are as follows: vestibular 83222 (4453–890217); geniculate 4307 (311–79518); cochlear 306 (86–32864); and trigeminal 111 (30–20257).

Table 1 HSV copy number per 10^5 cells

Subject	*5*	*7*	*C*	*V*
1	875	4526	32864	19777
2R	121	4904	5450	198912
2L	20257	633	217	158631
3R	100	4545	101	21524
3L	54	8859	189	283301
4R	2206	1273	1397	16499
4L	263	3034	395	4453
5	44	311	109	367955
6R	78	732	575	6144
6L	125	79518	807	8121
7	54	4087	139	890217
8	30	6956	86	144921

5 = trigeminal; 7 = geniculate; C = cochlear; V = vestibular.

Table 2 Average copy number and concentration/sample

Ganglion	*HSV*	*RNase P*	*DNA concentration*
Trigeminal (N = 50)	229	40382	133.3 ng
Geniculate (N = 47)	142	4311	14.2 ng
Cochlear (N = 46)	84	10536	34.8 ng
Vestibular (N = 47)	391	969	3.2 ng

N = number of samples tested.

The cycle threshold measurements for a given specimen displayed little variation on repetitive testing, producing consistent relative estimates of viral load. The measured RNase P gene copies per specimen did vary among the different ganglia (Table 2). The trigeminal ganglion had substantially greater cellular RNase P gene copies per specimen than the other ganglia ($P < .001$). For all ganglia, there was no significant correlation ($r = -.1$, $P = .5$) between RNase P copy number per specimen and HSV copy number per specimen, indicating a lack of competition or interference between the HSV and RNase P assays. A significant correlation would imply a relationship between the amount of input DNA and the ability to detect HSV.

There was no correlation of HSV viral load between ganglia from the same individual. Bivariate linear regression among all possible ganglion pairs showed variable positive and negative correlations though none were significant (all $P > .4$). This lack of association suggests viral load within a given ganglion is independently determined and not linked to a prerequisite high viral load in another ganglion.

Discussion

Study of HSV in human ganglia requires consideration of host-virus interaction over the course of one's lifetime. In contrast to animal models, exposure to HSV in humans will occur on multiple occasions, including autoinoculation in those individuals that manifest reactivation of latent virus. HSV is an efficient neuronal tracer and can be recovered from various central nervous system (CNS) sites after peripheral inoculation in animal models (Sugita *et al*, 1995). In human subjects, decades pass between inoculation and eventual study of ganglia or CNS tissue. Thus, one would expect that the virus could trace multiple neuronal pathways. Infection of the vestibular ganglion is postulated to occur via vestibulofacial anastomoses (Hirata *et al*, 1993; Vrabec and Payne, 2001). HSV has been recovered from both the vestibular nuclei and labyrinth, indicating further spread from this location (Arbusow *et al*, 2000,

2001). Involvement of the cochlear ganglion could either be via efferent projections from the brainstem or by direct inoculation of cochlear tissues via the labyrinthine fluids.

Prior estimates of the percentage of HSV infected neurons in human ganglia are no greater than 5% (Croen *et al*, 1987; Cai *et al*, 2002). In contrast, animal models displaying efficient reactivation may have 30% or more of neurons testing positive for HSV (Sawtell, 1998; Chen *et al*, 2002). However, it is difficult to directly compare the data from human and animal studies for many reasons. In contrast to the controlled laboratory setting, examination of autopsy subjects introduces variables such as different virus strain, inoculum titer, history of clinical reactivation, and time between initial infection and removal of the ganglia. Additional error can be introduced by incorrect assumptions needed to extrapolate data to the cellular level, including percentage of infected neurons, and ratio of neurons to supporting cells.

Prior studies of human trigeminal ganglia using quantitative polymerase chain reaction (PCR) methods reached similar estimates of HSV viral load (Pevenstein *et al*, 1999; Cohrs *et al*, 2000). In each, spectrophotometry was used to standardize an amount of template DNA for each sample. A measured quantity of viral copies is then expressed relative to the amount of template DNA. Pevenstein *et al* (1999) estimated the amount of DNA per cell to be 15.6 pg based on their experimental data. In this report, we use the theoretical amount of DNA/diploid cell of 6.6 pg (Si *et al*, 2003). Thus, to compare our data with Pevenstein *et al* (1999), a correction factor (6.6/15.6) is applied to their reported mean of 2902 copies per 10^5 cells, giving a new estimate of 1227 HSV copies per 10^5 cells (estimated range 22–4320). To compare our data to Cohrs *et al* (2000), multiply the number of HSV copies per 100 ng DNA in their report by 6.6 to derive a mean of 1343 HSV copies per 10^5 cells (range 151–5009). Finally, Cai *et al* (2002) defined a range of 2 to 50 copies of HSV per infected neuron, and detected HSV in 3% of neurons analyzed. To extrapolate these numbers to copies per 10^5 cells, we assume a 100:1 ratio of supporting cell/neuron (LaGuardia *et al*, 2000). This gives a range of 60 to 1500 HSV copies per 10^5 cells. Our data for the trigeminal ganglion (mean 2017 HSV copies per 10^5 cells, range 30–20257) display considerable overlap with the reported measurements of HSV viral load. Alternatively, direct comparison is possible between the trigeminal ganglia in this report and the HSV positive ganglia in Cohrs *et al* (2000), finding no significant difference in HSV copies per 10^5 cells (t test, $P = .58$). The reports cited all find wide variations in viral load within human ganglia, often over 2 orders of magnitude.

The estimation of viral load using the quantitative PCR technique described here will understate the amount of viral copies per ganglion cell because cell types are not differentiated in this method. Therefore, supporting cell DNA is included in the denominator when expressing the viral load per human genome equivalent in this study. Correction for this effect is difficult, as the number of supporting cells per neuron is probably different among the ganglia examined. The average neuronal cell count in cranial nerve ganglia is well established. Estimates for each ganglion are as follows: trigeminal, 27000; geniculate, 2100; cochlear, 35000; and vestibular, 20000 (Ball *et al*, 1982; Gacek, 1998; Velazquez-Villasenor *et al*, 2000; Tang *et al*, 2002). However, the number of supporting cells per neuron is uncertain. In the trigeminal ganglion, it is estimated that there are 100 supporting cells per neuron (LaGuardia *et al*, 2000). There are no published estimates for the other ganglia, therefore some assumptions must be made. The geniculate ganglion is similar to the trigeminal in that it contains sensory and motor neurons, therefore a supporting cell/neuron ratio of 100:1 seems plausible. If the supporting cell ratio is the same, there is no significant difference in viral load between the geniculate and trigeminal ganglia. The vestibular and cochlear ganglia appear to have fewer supporting cells on light microscopy. The supporting cell/neuron ratio in these ganglia may be as low as 3:1. Thus, a difference in supporting cell ratios cannot be excluded as an explanation for the difference in viral load among ganglia. Comparative studies in human ganglion specimens are relatively rare. A difference in prevalence of viral DNA in trigeminal versus spinal ganglia in humans has been reported (Mahalingham *et al*, 1992). But no other quantitative studies comparing different ganglia are available to corroborate the differences reported here.

A difference in viral load according to ganglion supports the concept of variable susceptibility of diverse neuronal populations to viral infection, latency, and reactivation. The neurons in these cranial nerve ganglia differ in function, cellar architecture, and neurotransmitters both within and among ganglia (Grigaliunas *et al*, 2002). Expression of cellular factors that modulate HSV infection represents another way to distinguish between neurons and define an "HSV-sensitive" phenotype. For example, latency-associated transcript expression, indicating latent HSV infection with a cell, is enhanced in neurons immunoreactive to monoclonal antibodies SSEA3 and A5, whereas those immunoreactive to KH10 enhance productive infection (Yang *et al*, 2000). The receptor nectin-1, a putative mediator of HSV entry into the cell, is expressed strongly in sensory neurons and much less in motor neurons (Mata *et al*, 2001). Expression of nuclear cdk2 is associated with increased potential for HSV reactivation (Schang *et al*, 2002). Finally, distinct neuronal populations are capable of activating HSV immediate-early genes ICP0 and ICP27 even in the absence of viral infection, including neurons in the vestibular nuclei (Loiacono *et al*, 2002). These studies indicate that multiple cellular factors can influence the course of HSV infection

within a given neuron. Consequently, it is naïve to assume that the distribution of "HSV-sensitive" neurons will be the same in all cranial nerve ganglia.

Surveys from autopsy subjects can provide population estimates of prevalence of infected neurons and viral load within a ganglion. The hypothesis that viral load is a determinant of reactivation is appealing, but expects a direct correlation between viral load and probability of reactivation (Sawtell, 1998). The present study finds viral load varying over several orders of magnitude within each ganglion and between different cranial nerve ganglia. Of particular interest is the lack of correlation of the HSV viral load among ganglia from the same individual. Presumably, if infecting strain and inoculum titer were critical to determining viral load in a ganglion, an individual exposed to a particularly virulent strain of HSV in an unusually high titer would reflect this in all ganglia surveyed. Thus, the lack of correlation suggests that viral strain and inoculum titer are less significant than neuronal phenotype in determining the course of HSV infection.

Human subjects with clinical symptoms of HSV reactivation display wide variations in age of onset, frequency of symptoms, and ganglion involved. Reactivation within the distribution of the trigeminal nerve is the most commonly recognized, presenting as herpes labialis or herpetic keratitis. If viral load were the most important factor in predicting reactivation in humans, one would expect to find the highest viral load in the trigeminal ganglion. This study seems to contradict the correlation between viral load and reactivation demonstrated in animal models. There are several potential explanations for this discrepancy. First, assumptions about supporting cell ratios could be incorrect resulting in a statistical error. Second, HSV reactivation within the distribution of the vestibular ganglion may be clinically under-ascertained. This is quite possible, as there are no routine clinical techniques to visualize, culture, or image the inner ear in a manner that could confirm HSV reactivation. Finally, the molecular mechanisms that determine latency and reactivation could be independent, allowing speculation that trigeminal neurons contain greater levels of cellular factors necessary for reactivation than those in the vestibular ganglion. Future studies are needed to demonstrate a distinct difference in reactivation efficiency among neurons from the various ganglia.

In summary, HSV viral load varies significantly among cranial nerve ganglia and is greatest in the vestibular ganglion. The present study matched ganglia within the same individual, thus the differences imply that susceptibility to HSV infection is determined at the cellular level. Future investigation is directed at further characterization of differences in neuronal populations among cranial nerve ganglia with regard to HSV infection and reactivation.

Methods

The trigeminal, geniculate, vestibular (Scarpa's), and cochlear (spiral) ganglia were collected from 20 willed-body donors frozen within 48 h of death by protocol. Each side of the head is counted as a single dissection, offering the potential for 40 sets of ganglia. In four instances, a complete set of ganglia was not obtained, leaving 36 sets for study. The clinical history regarding symptoms of HSV-mediated disease is unknown in all cases. Dissections were performed following variable storage intervals. After dissection, ganglia were stored at −80°C. DNA was extracted from each specimen using the QIAamp DNA Mini Kit, according to manufacturer's guidelines (Qiagen). Initial screening for the presence of HSV used previously published methods (Vrabec and Payne, 2001). Overall, the prevalence of HSV DNA according to ganglion was trigeminal, 50%; geniculate, 57%; vestibular, 81%; and cochlear, 85%. Twelve ganglion sets included in this study displayed HSV

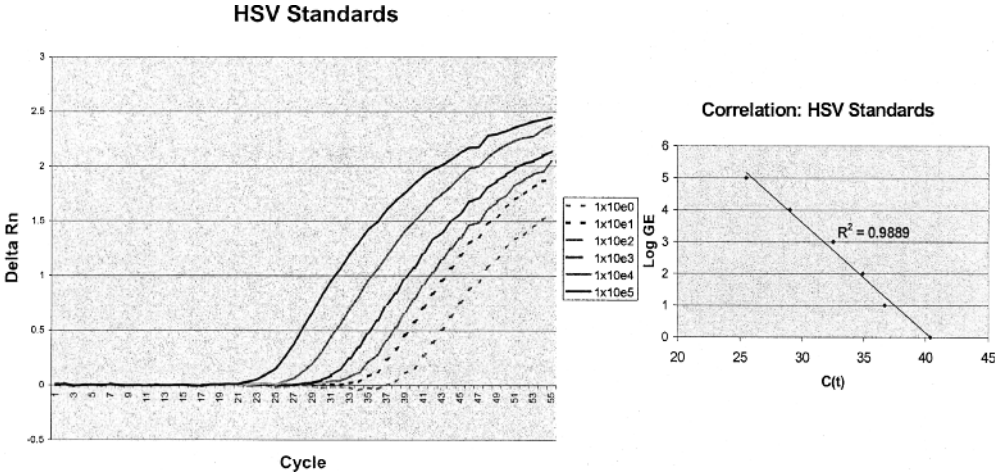

Figure 1 Performance of HSV standards. The graph on the left displays the amplification plots at HSV concentrations from 1 × 10e0 to 1 × 10e5. The graph on the right displays correlation between HSV concentration and measured cycle threshold.

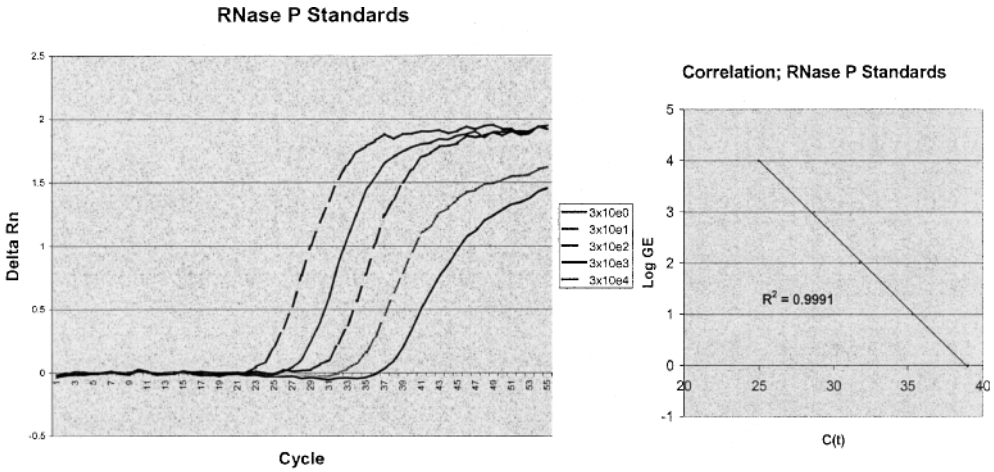

Figure 2 Performance of RNase P standards. The graph on the left displays the amplification plots at RNase P concentrations from 3 × 10e0 to 3 × 10e4. The graph on the right displays correlation between RNase P concentration and measured cycle threshold.

DNA in the trigeminal, geniculate, vestibular, and cochlear ganglia on the same side in the same individual. Matching the ganglia in this manner controls for inoculum titer and host susceptibility across each set.

A duplex reaction was designed to quantify HSV genome copy number and the amount of cellular DNA in each well. Custom made primers and probes are directed at the HSV DNA polymerase gene (UL30). The sequence of the primer/probe set is as follows: forward 5′-TCCTCACCGCCGAACTGA-3′; reverse 5′-CCAGGCGCTTGTTGGTGTA-3′; and probe 5′-AGACACCCGCGCGC-3′. A commercially available primer/probe set for amplification of the single-copy cellular RNase P gene is used to quantify the amount of genomic DNA per sample (Applied Biosystems). The two probes are labeled with different reporter dyes, allowing differentiation of PCR products according to emission wavelength. The reaction mixture is composed of 1 μl of sample DNA, 2× TaqMan Universal PCR Master mix, 250 nM of the HSV primers and probe, and 1× of the RNase P primers and probe. Specimens were run on the ABI 7000 sequence detection system (Applied Biosystems). Cycling parameters are 95° for 10 min, followed by 45 cycles of 95°C for 15 s, and 60°C for 60 s. Each specimen was tested at least three times. Multiple negative controls were included in each run, consisting of HSV-free genomic DNA and multiple wells containing the master mix only.

Table 3 Mean cycle thresholds

HSV copies	10^5	10^4	10^3	10^2	10^1	10^0
HSV (N = 11)	26.29	30.24	34.32	38.07	40.19	41.56
Standard deviation	1.06	1.27	1.16	1.62	1.84	1.9
RNase P copies		10^4	10^3	10^2	10^1	10^0
DNA (N = 11)		25.05	28.64	31.96	36.34	39.67
Standard deviation		1.64	1.54	1.41	1.95	2.15

HSV = HSV standards; DNA = genomic DNA standards; N = number of trials.

Quantitative viral standards are composed of purified viral DNA (HSV 17 syn+ strain) diluted in human genomic DNA. Serial dilutions of the stock HSV DNA results in standards containing 10^5 to 10^0 copies of the HSV genome mixed with 10 ng/μl of genomic DNA. Genomic DNA standards are created with serial dilutions of Epstein-Barr virus (EBV)-transformed

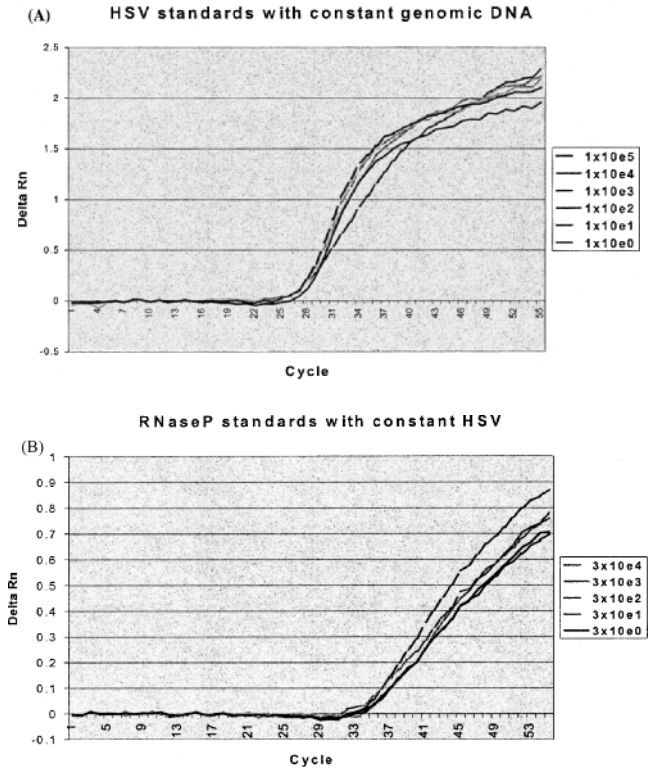

Figure 3 (A) HSV standards with constant 10 ng/μl human DNA added. (B) DNA standards with constant 100 copies HSV added. Both graphs show reproducible cycle thresholds for the constant despite wide variation in the competing template.

lymphocyte DNA (ATCC) to produce reference samples containing 3×10^4 to 3×10^0 copies of the RNase P gene. A linear correlation is expected between cycle threshold and log concentration for each set of standards ($r^2 > .95$). Runs that do not exhibit adequate correlation between standards or that include positive results in negative controls are discarded. The assay described is capable of detecting less than 10 copies of both HSV and RNase P (Figures 1 and 2).

Primer concentrations for the duplex assay were chosen to provide the most efficient (lowest) cycle thresholds for HSV and DNA standards. Multiple combinations of HSV primer amounts ranging from 50 to 500 nM and RNase P primers from $0.2\times$ to $1\times$ were tested in order to select the most robust mix. Cycle thresholds in the duplex assay were comparable to those obtained when running each primer set independently. Standards showed minimal variability in absolute cycle threshold on repetitive trials, as depicted in Table 3. Accuracy of the quantification was confirmed by measuring the cycle thresholds of the HSV standards with addition of varying amounts of human DNA, and by testing the genomic DNA standards with addition of varying amounts of viral DNA (Figure 3A, B).

Copy number for HSV genomes and RNase P genes per specimen are determined by extrapolation of cycle threshold to standard curves. The ratio of HSV copies to RNase P copies is calculated for each specimen. Averaging of repetitive trials provides the mean value for each specimen. The HSV/RNase P ratio is multiplied by 2×10^5 to give the copy number per 10^5 cells. Throughout this report, the amount of DNA per human diploid cell is assumed to be 6.6 pg (Si *et al*, 2003). Therefore in order to calculate the amount of DNA per sample, the measured RNase P copy number is multiplied by 3.3 pg. Copy number was compared and correlation calculated among ganglia using ANOVA. Statistical computations were done using the software package SPSS 10.0 (SPSS).

References

Amin MR, Koufman JA (2001). Vagal neuropathy after upper respiratory infection: a viral etiology? *Am J Otolaryngol* **22:** 251–256.

Arbusow V, Strupp M, Wasicky R, Horn AK, Schulz P, Brandt T (2000). Detection of herpes simplex virus type 1 in human vestibular nuclei. *Neurology* **55:** 880–882.

Arbusow V, Theil D, Strupp M, Mascolo A, Brandt T (2001). HSV-1 not only in human vestibular ganglia but also in the vestibular labyrinth. *Audiol Neurootol* **6:** 259–262.

Ball MJ, Nuttall K, Warren KG (1982). Neuronal and lymphocytic populations in human trigeminal ganglia: implications for ageing and for latent virus. *Neuropathol Appl Neurobiol* **8:** 177–187.

Baringer JR, Swoveland P (1973). Recovery of herpes simplex virus from human trigeminal ganglions. *New Engl J Med* **288:** 648–650.

Bastian FO, Rabson AS, Yee CL, *et al* (1972). Herpesvirus hominis: isolation from human trigeminal ganglion. *Science* **178:** 306–307.

Cai GY, Pizer LI, Levin MJ (2002). Fractionation of neurons and satellite cells from human sensory ganglia in order to study herpesvirus latency. *J Virol Methods* **104:** 21–32.

Carr DJ, Harle P, Gebhardt BM (2001). The immune response to ocular herpes simplex virus type 1 infection. *Exp Biol Med (Maywood)* **226:** 353–366.

Chen XP, Mata M, Kelley M, Glorioso JC, Fink DJ (2002). The relationship of herpes simplex virus latency associated transcript expression to genome copy number: a quantitative study using laser capture microdissection. *J NeuroVirol* **8:** 204–210.

Cohrs RJ, Randall J, Smith J, Gilden DH, Dabrowski C, van Der Keyl H, Tal-Singer R (2000). Analysis of individual human trigeminal ganglia for latent herpes simplex virus type 1 and varicella-zoster virus nucleic acids using real-time PCR. *J Virol* **74:** 11464–11471.

Croen KD, Ostrove JM, Dragovic LJ, Smialek JE, Straus SE (1987). Latent herpes simplex virus in human trigeminal ganglia. Detection of an immediate early gene "anti-sense" transcript by in situ hybridization. *N Engl J Med* **317:** 1427–1432.

Furuta Y, Takasu T, Sato KC, Fukuda S, Inuyama Y, Nagashima K (1992). Latent herpes simplex virus type 1 in human geniculate ganglia. *Acta Neuropathol* **84:** 39–44.

Gacek RR (1998). On the duality of the facial nerve ganglion. *Laryngoscope* **108:** 1077–1086.

Gacek RR, Gacek MR (2002). The three faces of vestibular ganglionitis. *Ann Otol Rhinol Laryngol* **111:** 103–114.

Gesser RM, Valyi-Nagy T, Fraser NW, Altschuler SM (1995). Oral inoculation of SCID mice with an attenuated herpes simplex virus-1 strain causes persistent enteric nervous system infection and gastric ulcers without direct mucosal infection. *Lab Invest* **73:** 880–889.

Grigaliunas A, Bradley RM, MacCallum DK, Mistretta CM (2002). Distinctive neurophysiological properties of embryonic trigeminal and geniculate neurons in culture. *J Neurophysiol* **88:** 2058–2074.

Hirata Y, Sugita T, Gyo K, Yanagihara N (1993). Experimental vestibular neuritis induced by herpes simplex virus. *Acta Otolaryngol Suppl (Stockh)* **503:** 79–81.

LaGuardia JJ, Cohrs RJ, Gilden DH (2000). Numbers of neurons and non-neuronal cells in human trigeminal ganglia. *Neurol Res* **22:** 565–566.

Liedtke W, Opalka B, Zimmermann CW, Lignitz E (1993). Age distribution of latent herpes simplex virus 1 and varicella-zoster virus genome in human nervous tissue. *J Neurol Sci* **116:** 6–11.

Loiacono CM, Myers R, Mitchell WJ (2002). Neurons differentially activate the herpes simplex virus type 1 immediate-early gene ICP0 and ICP27 promoters in transgenic mice. *J Virol* **76:** 2449–2459.

Maggioncalda J, Mehta A, Su YH, Fraser NW, Block TM 1996. Correlation between herpes simplex virus type 1 rate of reactivation from latent infection and the number of infected neurons in trigeminal ganglia. *Virology* **225:** 72–81.

Mahalingam R, Wellish MC, Dueland AN, Cohrs RJ, Gilden DH (1992). Localization of herpes simplex virus and varicella zoster virus DNA in human ganglia. *Ann Neurol* **31:** 444–448.

Mata M, Zhang M, Hu X, Fink DJ (2001). HveC (nectin-1) is expressed at high levels in sensory neurons, but not in motor neurons, of the rat peripheral nervous system. *J NeuroVirol* **7:** 476–480.

Murakami S, Mizobuchi M, Nakashiro Y, Doi T, Hato N, Yanagihara N (1996). Bell palsy and herpes simplex virus: identification of viral DNA in endoneurial fluid and muscle. *Ann Intern Med* **124:** 27–30.

Pevenstein SR, Williams RK, McChesney D, Mont EK, Smialek JE, Straus SE (1999). Quantitation of latent varicella-zoster virus and herpes simples virus genomes in human trigeminal ganglia. *J Virol* **73:** 10514–10518.

Sawtell NM (1997). Comprehensive quantification of herpes simplex virus latency at the single cell level. *J Virol* **71:** 5423–5431.

Sawtell NM (1998). The probability of in vivo reactivation of herpes simplex virus increases with the number of latently infected neurons in the ganglia. *J Virol* **72:** 6888–6892.

Sawtell NM, Poon DK, Tansky CS, Thompson RL (1998). The latent herpes simplex virus type 1 genome copy number in individual neurons is virus strain specific and correlates with reactivation. *J Virol* **72:** 5343–5350.

Schang LM, Bantly A, Schaffer PA (2002). Explant-induced reactivation of herpes simplex virus occurs in neurons expressing nuclear cdk2 and cdk4. *J Virol* **76:** 7724–7735.

Schulz P, Arbusow V, Strupp M, Dieterich M, Rauch E, Brandt T (1998). Highly variable distribution of HSV-1 specific DNA in human geniculate, vestibular and spiral ganglia. *Neurosci Lett* **252:** 139–142.

Si HX, Tsao SW, Poon CS, Wang LD, Wong YC, Cheung AL (2003). Viral load of HPV in esophageal squamous cell carcinoma. *Int J Cancer* **103:** 496–500.

Sugita T, Murakami S, Yanagihara N, Fujiwara Y, Hirata Y, Kurata T (1995). Facial nerve paralysis induced by herpes simplex virus in mice: an animal model of acute and transient facial paralysis. *Ann Otol Rhinol Laryngol* **104:** 574–581.

Tang Y, Lopez I, Ishiyama A (2002). Application of unbiased stereology on archival human temporal bone. *Laryngoscope* **112:** 526–533.

Tenser RB, Dawson M, Ressel SJ, Dunstan ME (1982). Detection of herpes simplex virus mRNA in latently infected trigeminal ganglion neurons by in situ hybridization. *Ann Neurol* **11:** 285–291.

Thompson RL, Sawtell NM (2000). Replication of herpes simplex virus type 1 within trigeminal ganglia is required for high frequency but not high viral genome copy number latency. *J Virol* **74:** 965–974.

Velazquez-Villasenor L, Merchant SN, Tsuji K, Glynn RJ, Wall C 3rd, Rauch SD (2000). Temporal bone studies of the human peripheral vestibular system. Normative Scarpa's ganglion cell data. *Ann Otol Rhinol Laryngol Suppl* **181:** 14–19.

Vrabec JT (2003). Herpes simplex virus and Meniere's disease. *Laryngoscope* **113:** 1431–1438.

Vrabec JT, Molina CP, West B (2000). Herpes simplex viral laryngitis. *Ann Otol Rhinol Laryngol* **109:** 611–614.

Vrabec JT, Payne D (2001). Prevalence of herpesviruses in cranial nerve ganglia. *Acta Otolaryngol* **121:** 831–835.

Warren KG, Brown SM, Wroblewska Z, Gilden D, Koprowski H, *et al* (1978). Isolation of latent herpes simplex virus from the superior cervical and vagus ganglions of human beings. *New Engl J Med* **298:** 1068–1069.

Yang L, Voytek CC, Margolis TP (2000). Immunohistochemical analysis of primary sensory neurons latently infected with herpes simplex virus type 1. *J Virol* **74:** 209–217.