Iranian Journal of Virology 2015;9(3): 6-14

Original Article

Prevalence of Herpes Simplex Virustype-1, 2 and Varicella Zoster Virus (VZV) in Eye Infection

Omidian J[1] , Sheikhi-Shooshtari F[1*], Fazeli M[2]

1. Department of Ophthalmology, Faculty of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran.
2. WHO Collaborating Center for Reference and Research on Rabies, Pasteur Institute of Iran, Tehran, Iran.

Abstract

Background and Aims: Herpes simplex virus (HSV) is a common cause of corneal disease and is the leading infectious cause of corneal blindness among developed nations. This study is aimed to provide an estimation of the incidence of Herpes Simplex Virustype-1, 2(HSV1, 2) and Varicella Zoster Virus (VZV) in tears and swab from eye infection by polymerase chain reaction (PCR) in eye disease in a suspected community.

Materials and Methods: Fifty subjects without signs of ocular herpetic disease enrolled in the study. Serum samples from all subjects were tested for HSV IgG antibodies by enzyme-linked immunosorbent assay (ELISA). Subjects were instructed to collect tear samples by touching the inner surface of the lower eyelid with an individually wrapped, sterile cotton swab and to place the swab immediately into labeled sterile tubes. Swabs were kept at 4°C until processed. Nucleic acid was extracted from the samples and PCR-amplified for HSV DNA.

Results: Among 50 samples, 3 samples were refused because internal controls were negative. HSV infection was established in 10% (5 out of 50) of all patients. The prevalence of HSV infection in patients with no clinical suspicion of herpetic keratitis was 6%. Histopathologic evaluation revealed that among samples with positive PCR results, 100% had evidence of inflammation, 55% had giant cells, 39% had necrosis, 59% had vascularization, 67 % had ulcer and 100% of them had inclusion bodies.

Conclusions: Because some of the patients with no clinical suspicion of herpes infection were found positive, we suggest that HSV to be considered as one of the underlying etiologies in any patient with corneal scar and keratitis. Therefore, performing further diagnostic methods, including PCR and histopathology, is mandatory to clearly understand the infection.

Keywords: Herpes Simplex Virus, HSV-1, HSV-2, VZV, Tear, eye infection Polymerase Chain Reaction.

Introduction

Herpes simplex virus types 1 (HSV-1) and 2 (HSV-2) belong to the same herpes virus family, Herpesviridae (1, 2). Alphaherpesvirinae, a subfamily of Herpesviridae, is a common causative agent of human virus infection and includes herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) and varicella-zoster virus (VZV). Although they are famous for resulting vesicles in the skin, the clinical manifestations which involve other areas than cutaneous area are more intrested (2, 3). Herpes simplex virus (HSV) infections are very common worldwide. Clinically, herpes viruses display a range of diseases. Common HSV infections involve skin and/or mucus affecting face and mouth,

[*] **Corresponding author:** Fariba Sheikhi-Shooshtari. Department of Ophthalmology, Faculty of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran.

[DOI: 10.21859/isv.9.3.6]

genitalia and other body parts (7). Also, these viruses can attack the eyes and the central nervous system (CNS) (5). Lesions caused by the herpes virus were first documented by Hippocrates (7). By adulthood, 70-90% of people would have HSV-1 antibodies. HSV-2 is predominantly spread through genital contact and sexual activity (2). HSV-1, and HSV-2 and VZV, which belong to the Herpesviridae family, are morphologically very similar, with large double stranded linear deoxyribonucleic acid (DNA) genomes. These Viruses are related with a variety of clinical signs and symptoms of several organs, including central nervous system (CNS) diseases, skin lesion, eye and keratoconjunctivitis. In immunocompromised patients, the viruses may also trigger severe clinical appearance (6). Herpes viruses cause various ocular diseases which are considered as one of the main causes of eye infection etiology and blindness worldwide. Herpetic stromal keratitis (HSK), as one of the major forms of ocular involvement of herpes viruses, is still one of the major causes of corneal blindness and visual loss (1). HSK infection is one of the known viral keratitis and the main cause of blindness and eye morbidity in developing countries. Eye infection is a latent infection in the body, mainly in the trigeminal ganglia and can be revived by fever, trauma, stress, immunosuppressive agent or exposure to ultraviolet radiation. The prevalence of ocular HSV disease has been estimated at 5.9 to 7.20 cases per 100000 annually, as reported in some countries (5). Conjunctivitis is one of the most common cause of ocular morbidity in the world. Microbial agents as well as allergy and trauma are the most common etiological factors for conjunctivitis. Viral conjunctivitis in their early stages cannot be differentiated clinically from each other and also from bacterial or allergic conjunctivitis. Among the viral agents, Human Adenoviruses (HAdV), HSV-1, HSV-2, VZV are the important recognized etiology of this disease (7). According to the studies on HSK in Iran, the prevalence of this infection among HSK sufferers was relatively varied between 30% - 88% (5). After the primary infection, the virus remains latent in the nervous ganglia, for later reactivation. HSV-1 becomes latent in neural ganglions including trigeminal and superior cervical ganglions in which the virus intermittently reactivates from latency with or without explicit stimulations. Patients who have frequent episodes of reactivation eventually developed irreversible corneal stromal swelling, neovascularization, and scarring with severe visual loss (1, 28). Reactivation and asymptomatic shedding occur both for HSV-1 and HSV-2 post infection (2). HSV is endemic in every human society and conjunctivitis caused by this virus has high morbidity and potentially are blinding. Eye infection caused by VZV usually when it became reactivated in the ophthalmic division of the trigeminal nerve. In this condition, a minority of patients may develop conjunctivitis, keratitis, uveitis, and ocular cranial-nerve palsies.HSV-2 is one of the most prevalent infections in the world (4). Herpes simplex virus type 2 (HSV-2) infections may give symptoms from the skin and mucous membranes and/or the nervous system. Neurological complications appear with or without mucocutaneous lesions (8) Primary diagnosis and treatment with acyclovir are essential in decreasing both mortality and the occurrence of neurological sequelae in surviving patients (8). HSV infection may be primary or recurrent (5). Diagnosis of herpetic disease of the cornea is made by using clinical history and can be confirmed by pathologic evaluation of the obtained corneal samples. Pathologic features showing corneal viral infection are not limited to stromal edema, but other symptoms such as acute and chronic inflammation, vascularization, necrosis, scar can be seen.8 Chronic cases presenting for surgery with corneal scar and insufficient clinical history pose a diagnostic challenge. In such cases inattention to herpetic etiology of the scar can lead to keratitis, relapse and treatment failure (1). Curing HSK is hard for ophthalmologists despite the existence of advanced diagnostic methods and impressive effect of antiviral drugs such as acyclovir (5). In the clinical laboratory, conventional methods for detecting HSV and VSV such as

[DOI: 10.21859/isv.9.3.6]

cell culture or direct immunofluorescent assay (DFA) have limitations such as slowness, insensitivity, and non-standardization in interpretation (3, 7) and newer methods are and nucleic acid detection. The latter is more sensitive and is not dependent on the presence of viable virus or the quality and presence of appropriately infected cells Early, rapid and reliable diagnosis of such infections, especially HSE and HSK, is very critical for patients' safety (5). However, the clinical manifestation of herpes keratitis is too varied to be diagnosed solely based on its clinical findings. Up until now, laboratory diagnostic tools have not been good enough to detect HSV definitively in patients with herpes keratitis (9). Some of the corneal scars are caused by latent HSV infection that has been detected by various methods such as immunohistochemistry and polymerase chain reaction (PCR). These methods are used to detect HSV-1 and HSV-2 antigens or DNA in latent and reactivated HSV infection. Furthermore, HSV-1 DNA in the cornea is quantified by real-time PCR, and the copy number in the cornea is correlated with the frequency of reactivation of herpetic keratitis (1). Many molecular biology laboratories use in-house PCR assays – techniques specifically developed by laboratories for the diagnosis of infectious diseases. However, the lack of standardization and the poor reproducibility of in-house techniques have been argued as limitations against their routine use as diagnostic methods (6). PCR has been shown in recent studies to be a rapid and reliable method for detecting HSV infection (10). Recent studies have shown that polymerase chain reaction (PCR) increases the rate of HSV detection in mucocutaneous swabs by 11–41% compared to virus culture (11). This method has also shown an increased sensitivity for the detection of HSV DNA in clinical specimens compared to antigen detection and culture methods (10). Whereas published protocols for herpes simplex virus (HSV) PCR do not always differentiate between HSV types, new technologies allow simultaneous detection and typing of HSV in a single reaction tube (11). Since HSV-1, HSV-2 and VZV are of the most prevalent infections in the world, and the clinical feasibility of PCR is still being debated in the ophthalmologic field because of the reported disadvantages mentioned above as well as more recent reports that have shown low sensitivity of herpes PCR in tears and epithelial cells, Therefore, the purpose of this study was to comparatively investigate the methodological efficacy of PCR assay in the detection of HSV in tears and to analyze other factors that affect the positive rate. This study assessed HSV-1,2 and VZV prevalence using the polymerase chain reaction (PCR) technique, as well as the factors associated with its infection.

Methods

Patients and sampling criteria. This study was approved by the medical ethics committee of the Medical University, Ministry of Health and Medical Education. The study confirms adherence to the Declaration of Helsinki. Consent to remaining in the research, obtained as soon as possible from the subject or a legally authorized representative. All patients informed for further testing on their samples. All patients with a previous history of HSV antibody were selected (n=50). A total of 50 affected eyes of 50 patients with clinically suspected HSK and 50 eyes from 50 healthy volunteers (control group) was entered into this study. Samples were obtained from all registered individuals. Sex and age matching performed for patients and healthy persons. All samples were collected and manipulated within maximum 1 hour at room temperature or Swabs were kept at 4°C for 1-3 hours until processed or aliquoted and stock at -70°C until later examination.

Samples. All samples were collected within maximum six hours to arrive at our laboratory and manipulated within maximum 1 hour at room temperature or Swabs were kept at 4°C for 1-3 hours until processed or aliquoted and stock at -70°C until later examination. Swabs were immersed in distilled water as eluent and resulted suspension was used for total DNA extraction. DNA was isolated using the QIAamp Tissue Kit (Qiagen company). Extracted DNA samples were dissolved in 200

[DOI: 10.21859/isv.9.3.6]

µl of sterile TRIS-EDTA, pH 8 and visualized by electrophoresis on 0.7% agarose gel.

DNA extraction. DNA extraction from the clinical specimens was performed using the QIAamp Tissue Kit (Qiagen company) as the manufacturer's instructions. Extracted DNA samples were dissolved in 200 µl of sterile TRIS-EDTA, pH 8 and visualized by electrophoresis on 0.7% agarose gel. The resulted extract was used for PCR for HSV; CMV and VZV.

Sensitivity of PCR. The sensitivity of the PCR was tested by serial tenfold dilutions of the extracted positive control DNA of both HSV -1 and 2 in sterile Milli Q water. PCR was performed on the diluted samples and the sensitivity was determined. The sensitivity of two nucleic acid extraction protocols was measured by determining PCRD50 as the highest dilutions of the sample that gave a 50% detection rate by PCR. To further evaluate the sensitivity of PCR on clinical specimens, we performed PCR analysis on dilutions of previously quantified stacks of patient-derived HSV particles. HSV-1 and HSV-2 stocks were subject to 2-fold serial dilution using distilled water as a diluent. To determine the maximum dilution of whole, virus particles detectable by the assay, the diluted samples were subjected to PCR. Purified HSV-1 and HSV-2 target DNA standard (provided by the kit) were found to be detectable at a minimum level of 10 copies in 4 out of 4 reactions, with the highest detected crossing point values being 25.97 for HSV-2 and 24.72 for HSV-1.

Specificity of PCR. Since high rates of false-positive negatively affect the interpretation of the PCR observed sensitivity, must first present the specificity results in the study. The specificity of the developed PCR test verifies by testing several DNA samples extracted from different sources including bacteria, Fungal over the intended PCR. The cross reactivity between the each of the primer sets, herpes group of viruses were also determined by testing the primers against varicella zoster virus DNA (Oka vaccine strain), cytomegalovirus DNA (AD169) and standard or laboratory isolate of EBV, HHV-6.

Validation of the type specific PCR with the isolates. DNAs of 10 random clinical isolates and the standard DNA of HSV 1 and 2 provided with a kit were extracted and PCR tests performed and the results were analyzed.

DNA sequencing. PCR products from the HSV-1, HSV-2, and VZV strains were purified with a QIAquick PCR purification kit (QIAGEN, Hilden, Germany) and used as templates for DNA sequencing reactions. The 50mL amplified products of the clinical samples were run on a 2% agarose gel and electrophoresed. The 272 bp amplified product was visualized in the UV transilluminator and the amplified product was cut using a sterile blade and transferred into a 1.5mL microfuge tube. The DNA was eluted from the agarose gel using the QIAquick DNA mini gel elution kit in a total of 10mL elution volume and the whole 10mL was provided to Tehran, Taleghani laboratory for sequencing. All the positive samples obtained in the PCR were sequenced to determine the specificity of the PCR and to rule out any false positivity.

POLYMERASE CHAIN REACTION. Primers for HSV-1, HSV-2, and VZV DNA were selected from published sequences and checked for correctness by computer assisted analysis (BLAST searches on European Molecular Biology Laboratory (EMBL), GenBank databases) (12, 13, 14). Primers selected for this study were shown at the follow. Selected primers for PCR were checked by Mega 4 NCBI (National Center for Biotechnology Information) and were based on alignments of different viral genome sequences from Gen Bank. Based on examination software, primer sets used in this study amplified approximately all known HSV and VZV variants. Gene Runner, NCBI Blast, Oligo analyzer3, and Oligo6 software were used to select and compare different sets of primers. In order to obtain the most appropriate combination, results of virtual amplifications were evaluated and different pairs of primers were selected as follows.

The following primer sequences to detect HSV-1 DNA were used in this study:

HSV1 – OS: 5'-AAT-CGC-GAA-CAT-CTA-CACCAC-3'

[DOI: 10.21859/isv.9.3.6]

HSV1 - IS: 5'-AAA-GCT-GTC-CCC-AAT-CCTCCC-3'
HSV1 - OA: 5'-CTG-CAG-ATA-CCG-CAC-CGTATT-3'
HSV1 - IA: 5'-CAT-CTT-CGA-CCG-CCA-TCCCAT-3'
The following primer sequences to detect HSV-2 DNA were used in this study:
HSV2 - OS: 5 GGACGAGGCCCGAAAGCACA 3
HSV2 - IS: 5 GGACGAGGCCCGAAAGCACA 3
HSV2 - OA: 5 CGGTGCTCCAGGATAAA 3
HSV2 - IA: 5 TCTCCGTCCAGTCGTTTATCTTC 3
The following primer sequences to detect VZV DNA were used in this study:
VZV-1: 5'-AAT-GCC-GTG-ACC-ACCAAG-TAT-AAT-3'
VZV-2: 5'-TAC-GGG-TCT-TGC-CGGAGC-TGG-TAT-3'
VZV-3: 5'-TCC-ATC-TGT-CTT-TGT-CTTTCA-C-3'
VZV-4: 5'-ATT-TTC-TGG-CTC-TAATCC-AAG-G-3'
All primer pairs were tested for the optimal Mg2+ concentration and annealing temperature (Table 3) based on references (12, 13, 14).
The amplification mixtures contained 100 ng of sense and antisense primers (CINNAGEN, COMPANY ,TEHRAN, IRAN), 0.2 mM of dATP, dCTP, dGTP, and dTTP, 1 unit of Taq DNA polymerase (FERMENTAS COMPANY, CAT NUMBER EP0402), 2.5 µl of extracted template DNA solution, [Mg2+] (FERMENTAS COMPANY, CAT NUMBER R0971) (Table 3), and final concentrations of PCR buffer (FERMENTAS COMPANY, CAT NUMBER B38), containing 0.15M (NH4)2SO4, 0.25M KCl, 0.2M NaCl, 0.1M TRIS.HCl, 20 mg/ml bovine serum albumin, pH 8.3, and distilled water in a total volume of 25 µl. The first product was amplified using nested primers. The PCR was performed in a thermal cyclers (Applied Biosystem, USA) as follows: the samples were denatured at 95°C for 4 minutes, 35 cycles of denaturation for 30 seconds at 95°C, primer annealing for 60 seconds at the optimal temperature, and elongation for 85 seconds at 72°C were performed. After the last cycle, samples were incubated for 10 minutes at 72°C for additional elongation and stored at 4°C. The risk of contamination in the PCR was minimized by careful handling of all material and separate rooms for the three stages of PCR were used, dedicated pipettes with plugged tips were used. Negative control samples were composed of sterile water and eye bank derived total cornea lysate. These samples were used in each round of DNA isolation and PCR analysis. To determine the maximum dilution of whole virus particles detectable by the assay, the diluted samples were subjected to PCR. Purified HSV-1 and HSV-2 target DNA standard (provided by the kit) were found to be detectable at a minimum level of 10 copies in 4 out of 4 reactions, with the highest detected crossing point values being 25.97 for HSV-2 and 24.72 for HSV-1. DNA standard was tested as positive controls. Half of the final PCR product was run on 1.8% agarose gel containing ethidium bromide and illuminated by UV light. After DNA sequencing, the nucleotide sequence was checked for correctness by computer assisted analysis (BLAST searches on EMBL, GenBank). The detection limits of each round of PCR were determined using known amounts of DNA, containing the HSV-1, HSV-2, or VZV specific fragments. In the nested HSV-1 DNA PCR 100 of template copies could be detected, in the HSV-2 DNA PCR 100 copies, and in the nested VZV DNA PCR 100 of template copies could be detected. No cross reactivity between each of the primer sets and positive controls for HSV-1, HSV-2, VZV, or human DNA was observed.

Statistical analysis. The statistical comparison of PCR results was analyzed using the SPSS ver. 19 (SPSS Inc. Chicago, IL). Descriptive analysis and Chi-square tests were used to explain the results and compare between the incidence of the infection using positive result of PCR assay in different age and sex groups. Significance was assigned to calculate p-values <0.05.

[DOI: 10.21859/isv.9.3.6]

Results

First, we analyzed the demographics of the enrolled patients. The mean age of the patients was 43.5 (19–71) for men and 36.5 (19–70) years in the female. The male to female ratio was 25/25. Fifty (100%) patients revealed a previous history of HSV disease, and 4 patients had used the antiviral agent within 12 months of the tear sampling. Next, we analyzed the total positive rate of tear PCR assay and the conditional positive rates, according to the methods used to collect the tears and the methods used to detect herpes. The total incidence rate of positive PCR was 10% (5 out of 50) in the tears. Finally, we investigated whether clinical factors affect the positive rates of tear PCR. The patients who presented with a typical eye lesion displayed higher positive rates of PCR than the patients with no lesion (p=0.006). There was no significant statistical difference in the positive PCR rates between the patients with previous herpes keratitis history and patients without previous history. Finally, the positive PCR rates were no greater in the patients without previous administration of anti-herpecidal medication than in the patients with the previous administration of the antiviral agent within 12 months before the sampling ($p < 0.001$).

Discussion

HSV1, HSV2 and VZV are the most common pathogens etiology of severe ocular and CNS infections (15, 16). Several in-house and commercially available platforms such as the "Lyra® Direct HSV1+2/VZV assay" (Quidel Germany GmbH, Germany), "RealStar® alpha Herpesvirus PCR" Kit (Altona Diagnostics, Germany) and "HSV-1 HSV-2 VZV R-gene®kit" (Argene/bioMerieux, France) are developed for detection of these viruses, but all of these tests are either not approved for all kinds of sample such as CSF or cannot be run with full automation (15-19). Golden methods for diagnosis and typing of HSV have included ELISA, IFA and isolation of viruses by cell culture. Although mentioned methods have been very practical in helping clinical

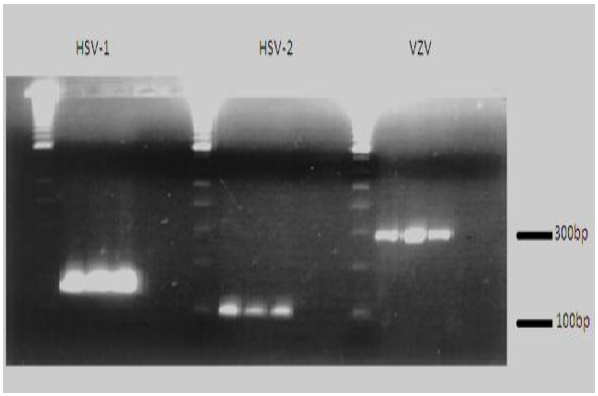

Fig. 1. Results of nested PCR to detect HSV-1, HSV-2, and VZV.

diagnosis, it has been revealed limitations of these methods. Both three assays are arduous and ponderous (20-23). We used a PCR method, for the detection of HSV-1, HSV-2, and VZV. The ability to produce a desired or intended result for PCR is determined by its specificity, efficiency (yield), and fidelity. A highly specific PCR will generate one and only one amplification product that is slightly and the intended target sequence (24). A positive test result in the our HSV-1 PCR was obtained in 10 out of 50 samples and anti-HSV antibody production was detectable in all cases. In this work, we determined the prevalence and diagnostic value of detecting a-HHV–specific DNA in human. HHV DNA was rarely detected in cornea donor tissues and in the excised corneas of patients without HK (25). The HSV-1 DNA load and presence in corneas was inversely correlated with graft survival in patients with HK (26, 27). The method was detecting each viral standard genome in reaction without cross-reaction in the presence of DNA extracted from the virus-negative plasma. Laboratory diagnostic testing of cornea specimens is of additional value in diagnosing cornea disease. The qualitative PCR and, more recently, quantitative PCR has mainly ameliorate the diagnostic sensitivity of etiologic agent in clinical specimens obtained from persons with suspected cases of infectious keratitis (28). As reported/showed earlier, HSV-1 was the most prevalent a-HHV identified in cornea tissues and was most frequently diagnosed in the corneas of patients with HK than in the corneas of patients without

[DOI: 10.21859/isv.9.3.6]

Table 1: Demographic data of sample patients

	***N* (%)**
Gender	
Male	25 (50)
Female	25 (50)
Race	
Iranian	**50(100)**
Age (mean)	
Male	43.5 (19–71)
Female	36.5 (19–70)
History of approved herpetic disease (*n* = 50)	
Ocular	0
Cutaneous(cold sores)	9
Genital	2
Education	
Less than high school	9(18)
High school	14(28)
Greater than high school	27(54)
HSV antibody	Positive 50(100)
	Negative 0(0)

HK. The low detection rate of HSV-2 and VZV in cornea tissues, as described by other investigators (25,29), may in order to a result of the different anatomical location of latent HSV-2, in comparison with HSV-1 and VZV, and of the relative low reactivation frequency of VZV, against HSV, in immunocompetent patients (25). In many clinical cases, a very rapid diagnosis is crucial for patients or organ survival, and PCR is being much faster than other methods. Viral DNA will detect by real-time PCR within 2.5 hours, which included DNA preparation from clinical specimens. The nested PCR assays take about 4-6 hours, making the real-time PCR assays 2-3 times faster than the nested assays (30, 31). In summary, the performed assays are suitable tools for the rapid diagnosis of HSV-1, HSV-2, and VZV infections in the laboratory. Due to the ease of use, the significant decrease in testing and handling time, and faster performance, PCR assays can be used in the laboratory, even in emergency patients in which fast diagnosis is crucial. The use of PCR for the diagnosis of severe disease has been well understood for HSV infections. The use of this highly sensitive technique has increased our understanding of the etiological role and mechanism of viruses in disease.

References

1. Asadi-Amoli F, Alaie-Gohar M, Shahsiah R. Detection of Herpes Simplex Virus in Corneal Buttons with Clinical Diagnosis of Corneal Scarring, Iranian Journal of Ophthalmology 2013;25(3):222-6.
2. Najafi S, Ghane M, Poortahmasebi V, Jazayeri SM, Yousefzadeh-Chabok S. Prevalence of Herpes Simplex Virus Infection in Patients With Relapsing-Remitting Multiple Sclerosis: A Case-Control Study in the North of Iran. Arch Clin Infect Dis. 2016;11(3):e36576.
3. Hong YJ, Lim MS, Hwang SM, Kim TS, Park KU, Song J, Kim EC. Detection of Herpes Simplex and Varicella-Zoster Virus in Clinical Specimens by Multiplex Real-Time PCRand Melting Curve Analysis. BioMed Research International. 2014, ID 261947, 5 p.
4. Caldeira TDM. Gonçalves, CV. Oliveira, GRD. Fonseca, TVD. Gonçalves, R. Amaral, CTD. Hora, VPD. Martinez AMB. Prevalence of herpes simplex virus type 2 and risk factors associated with this infection in women in southern brazil. Rev. Inst. Med. Trop. Sao Paulo. 2013;55(5):315-21.
5. Aliabadi N, Jamalidoust M, Asaei S, Namayandeh M, Ziyaeyan M. Diagnosing of Herpes Simplex Virus Infections in Suspected

[DOI: 10.21859/isv.9.3.6]

Table 2: Calculation of sensitivities of PCR or reverse transcription-PCR for detection of HSV-1, HSV-1 and VZV.

Virus	Log infectivity (TCID50/ml)[a]	Log NVP/ml[b]	Log PCRD50/ml[c]	NVP/TCID50	NVP/PCRD50	TCID50/PCRD50
HSV-1	7.475	9.43	8.05	57.5	18.4 [d]	0.345
HSV-2	8.05	10.051	9.2	63.25	6.325	0.115
VZV	5.75	9.545	9.2	2.29425	2.3	0.00115

[a] TCID50, TCID at which 50% of inoculated monolayers become infected.
[b] The number of virus particles (NVP) per milliliter was calculated from electron microscopic counts in parallel with counts of silicon beads by direct proportionality.
[c]PCRD50: 50% detection rate by PCR.

Table 3: PCR methods

PCR for	Primer pair	$[Mg^{2+}]$ (M)	Annealing temperature (°C)	Product size (base pairs)
HSV-1	OS, OA	0.002	60	507
	IS, IA	0.003	62	274
HSV-2	OS, OA	0.002	66	397
	IS, IA	0.002	66	
VZV	VZV1,VZV2	0.003	61	274
	VZV3, ZV4	0.003	60	

OS: Outer Sense/OA: Outer Antisense/IS: Internal Sense/IA: Internal Antisense

Patients Using Real-Time PCR. Jundishapur J Microbiol. 2015;8(2): e16727.
6. Rodrigues D, Paris F, Minuto-Paiva R. Minimum detection limit of an in-house nested-PCR assay for herpes simplex virus and varicella zoster virus. Revista da Sociedade Brasileira de Medicina Tropical. 2013;46(5):625-8.
7. Sohrabi M, Goodarzi Z, Saberfar E, Lashini H. The Prevalence of Viral Conjunctivitis in Patients Who Referred to Eye Specialist Hospitals in Tehran, Iran. Iranian Journal of Ophthalmology. 2014;26(1):29-32.
8. Franzen-Ro¨h E, Tiveljung-Lindell A, Grillner L, Aurelius E. Increased Detection Rate in Diagnosis of Herpes Simplex Virus Type 2. Meningitis by Real-Time PCR Using Cerebrospinal Fluid Samples. JOURNAL OF CLINICAL Microbiology. 2007;2516–20.
9. Lee SY, Kim MJ, Kim MK, Wee W. Comparative Analysis of Polymerase Chain Reaction Assay for Herpes Simplex Virus 1 Detection in Tear. Korean J Ophthalmol. 2013;27(5):316-21.
10.Gloria E, Kirsten A, Alvin Ch, Anderson P, Wernol M, Jennings C, et al. Comparison of real-time PCR with culture and EIA for the diagnosis of mucocutaneous infections with herpes simplex virus. New Zealand Journal of Medical Laboratory Science. 2006;60(3): p92.
11.Ramaswamy M, McDonald C, Smith M, et al. Diagnosis of genital herpes by real time PCR in routine clinical practice. Sexually Transmitted Infections. 2004;80(5):406-10.
12.Boer de JH, Verhagen C, Bruinenberg M, et al. Serologic and polymerase chain reaction analysis of intraocular fluids in the diagnosis of infectious uveitis. Am J Ophthalmol.1996;121:650–8.
13.Cantin EM, Chen J, McNeil J, et al. Detection of herpes simplex virus DNA sequences in corneal transplant recipients by polymerase chain reaction assays. Curr Eye Res. 1991;10:15–21.
14.Van Gelderen BE, Van der Lelij A, Treffers WF, Van der Gaag R. Detection of herpes simplex virus type 1, 2 and varicella zoster virus DNA in recipient corneal buttons. Br J Ophthalmol. 2000;84:1238-43.
15.Köller T, Kurze D, Lange M, et al. Implementation and Evaluation of a Fully Automated Multiplex Real-Time PCR Assay on the BD Max Platform to Detect and Differentiate Herpesviridae from Cerebrospinal Fluids. DeLuca NA, ed. PLoS ONE. 2016;11(4):e0153991.
16.Nipaporn S, Saovaluk S, Chulapan E, et al. Single tube multiplex real-time PCR for the rapid detection of herpesvirus infections of the central nervous system. Mol Cell Probes. 2011;25(2-3): 114–20.
17.Susan Bennett, William F. Carman, Rory N. Gunson. The development of a multiplex real-time PCR for the detection of herpes simplex virus 1 and 2, varizella zoster virus, adenovirus and Chlamydia trachomatis from eye swabs. J Virol Methods. 2013;189(1):143–7.
18.Daelynn R, Buelow M, et al. Comparison of two multiplexed PCR assays for the detection of HSV-1, HSV-2, and VZV with extracted and unextracted

[DOI: 10.21859/isv.9.3.6]

cutaneous and mucosal specimens. J Clin Virol. 2013;58(1):84–8.
19.B. Roizman R. The nine ages of herpes simplex virus. Whitley Herpes. 2001;8(1):23–7.
20.Singh A, Preiksaitis J, Ferenczy A, Romanowski B. The laboratory diagnosis of herpes simplex virus infections. The Canadian Journal of Infectious Diseases & Medical Microbiology. 2005;16(2):92-98.
21.Halstead DC, Beckwith DG, Sautter RL, Plosila L, Schneck KA. Evaluation of a rapid latex slide agglutination test for herpes simplex virus as a specimen screen and culture identification method. Journal of Clinical Microbiology. 1987;25(5):936-937.
22.Pandori MW, Lei J, Wong EH, et all. Real-Time PCR for detection of herpes simplex virus without nucleic acid extraction. BMC Infectious Diseases. 2006;6:104.
23.Hoffmann D, Jungkind G, Halle L, et al. Evaluation of two rapid methods for the detection of herpes simplex virus antigen in patient specimens. Ann Clin Lab Sci. 1985;15(5):418–27.
24.Cha R, Thilly W. Specificity, efficiency, and fidelity of PCR. PCR Methods Appl. 1993;3(3):18–29.
25.Farooq AV, Shukla D. Corneal latency and transmission of herpes simplex virus-1. Future virology. 2011;6(1):101-8.
26.Hill JM, Clement C. HSV-1 DNA in Human Corneas: What are the Virological and Clinical Implications? The Journal of infectious diseases. 2009;200(1):1-4.
27.Remeijer L, Duan R, Jessica M. et al. Prevalence and clinical consequences of herpes simplex virus type 1 DNA in human cornea tissues. J Infect Dis. 2009;200(1):11–9.
28.Gupta N, Tandon R. Investigative modalities in infectious keratitis. Indian Journal of Ophthalmology. 2008;56(3):209-13.
29.Kennedy DP, Clement C, Arceneaux RL, et all. Ocular HSV-1: Is the Cornea a Reservoir for Viral Latency or a Fast Pit Stop? Cornea. 2011;30(3):251-9.
30.Weidmann M, Meyer-König U, Hufert FT. Rapid Detection of Herpes Simplex Virus and Varicella-Zoster Virus Infections by Real-Time PCR. Journal of Clinical Microbiology. 2003;41(4):1565-8.
31.Speers DJ. Clinical Applications of Molecular Biology for Infectious Diseases.Clinical Biochemist Reviews. 2006;27(1):39-51.

[DOI: 10.21859/isv.9.3.6]