Available online on www.ijtpr.com
International Journal of Toxicological and Pharmacological Research 2013; 5(2): 42-46

ISSN: 0975-5160

Research Article

Ease of Dual Priming Oligonucleotide Technology for the Genotyping of Herpes Simplex Virus.

*[1]Narotam Sharma, [1]Satish.C.Nautiyal, [1]MD. Zohaib Ahmed, [3]Neha Saini, [2]Renu Dhar, [3]Harshita Verma, [3]Bhawana Bhatt, [3]Yogyata Jadli, [1]R.K. Singh

[1]Molecular Research Laboratory, Department of Biochemistry, Shri Guru Ram Rai Institute of Medical & Health Sciences, Patel Nagar, Dehradun-248001(Uttarakhand), India.
[2]Sai Institute of Paramedical and Allied Sciences, Dehradun (U.K), India.
[3]SGRR (P.G.) College, Pathribagh, Dehradun (U.K.), India.

ABSTRACT
Herpes simplex virus (HSV) encephalitis is a serious infection but diagnosis previously required brain biopsy in certain cases due to the low sensitivity of cerebrospinal fluid (CSF) culture and serology. PCR allows the detection of HSV DNA from CSF with 95% sensitivity thus avoiding invasive brain biopsy. HSV type 1and type 2 is highly contagious, and can infect skin, mouth and genital organs, both in male and females. Present study, includes the genotyping of HSV type 1 and 2 with the utilization of DPO (dual priming oligonucleotides) primers by conventional PCR. DPO PCR detected 2 out of 8 specimens with HSV infection. Though, study needs the trials of the technology on more number of specimens which can be useful as compared to serological or culture techniques for Herpes Simplex Virus.

Keywords: Genital Herpes, Dual priming, Polymerase chain reaction, Amplicons, Encephalitis.

INTRODUCTION

Sexually transmitted infectious agents (STI's) are more dynamic than other diseases prevailing in the community. Their epidemiological profile varies from country to country and from one region to another within a country, depending upon ethnographic, demographic, socioeconomic and health factors. The clinical pattern is also a result of the interaction among pathogens, the behaviors that transmit them and the effectiveness of preventive and control interventions[1]. Herpes simplex virus (HSV) has affected more than one third of the world's population and in developing nation like India it is believed that 60% of sexually active adults carries herpes viruses[2]. Acute persistent genital herpes was one of the first recognized opportunistic infection of acquired immune deficiency. In fact, about 60 to 85% of persons with HIV infection harbor HSV-2 antibodies[3]**.** Both Herpes Simplex Virus-1 (mostly produces most cold sores) and HSV-2 (which produces genital herpes) is ubiquitous and contagious[1]. HSV-1 is considered to be oral-facial herpes, commonly appearing on the lips and nacres as cold sores, transmitted mostly by oral lesions or secretions[4]**.**The primary HSV-1 infection does not usually produce symptoms, but if so, they can be very painful. Blisters forms on the lips, may also on the tongue produce too[5]. Painful open sores result from the blisters, and a yellowish membrane develops prior to healing. In children, the infection is accompanied by increased salivation and foul breath. HSV-2 is considered to be genital herpes, in which one in five people are correctly diagnosed[6]. The virus is transmitted mostly by sexual contact, and it is possible to spread it when one is feeling perfectly well. HSV is highly contagious and either type can affect the mouth or genitals. Primary infection occurs around the genital areas two to eight days after contracting with the virus[7]. Symptoms such as weakness, fever, headache, nerve pain, itching, lower abdominal pain, urinary difficulties, and yeast infections and vaginal discharge (in case of females) are clinical manifestations accompanying the eruptions on the skin[7].

Diagnosis: Genital herpes can be more difficult to diagnose than oral herpes, since most HSV-2-infected persons have no classical symptoms. Further confusing diagnosis, several other conditions resemble genital herpes, including fungal infection, lichen planus, atopic dermatitis, and urethritis[8]. Laboratory testing is often used to confirm a diagnosis of genital herpes. Laboratory studies includes; cell culture, direct fluorescent antibody (DFA), ELISA, Flow cytometry and nucleic acid based amplification tests.

Until recently, serological tests for antibodies detection for HSV were rarely useful to diagnosis and not routinely used in clinical practice. The older IgM serological assays could not differentiate between antibodies generated in response to HSV-1 or HSV-2 infection[9]. However, the new Immunodot glycoprotein G-specific (IgG) HSV test is more than 98% specific in discriminating HSV-1 from HSV-2 type[9]. It is the opinion of some modern medical professionals that the new IgG test should always be clinically preferred to the old IgM test, however not all doctors appear to be informed of the availability of the newer, reliable IgG tests. Biopsy procedures including skin biopsy, brain biopsy, although these procedures

*Author for correspondence: Email: sharmanarotam5@gmail.com

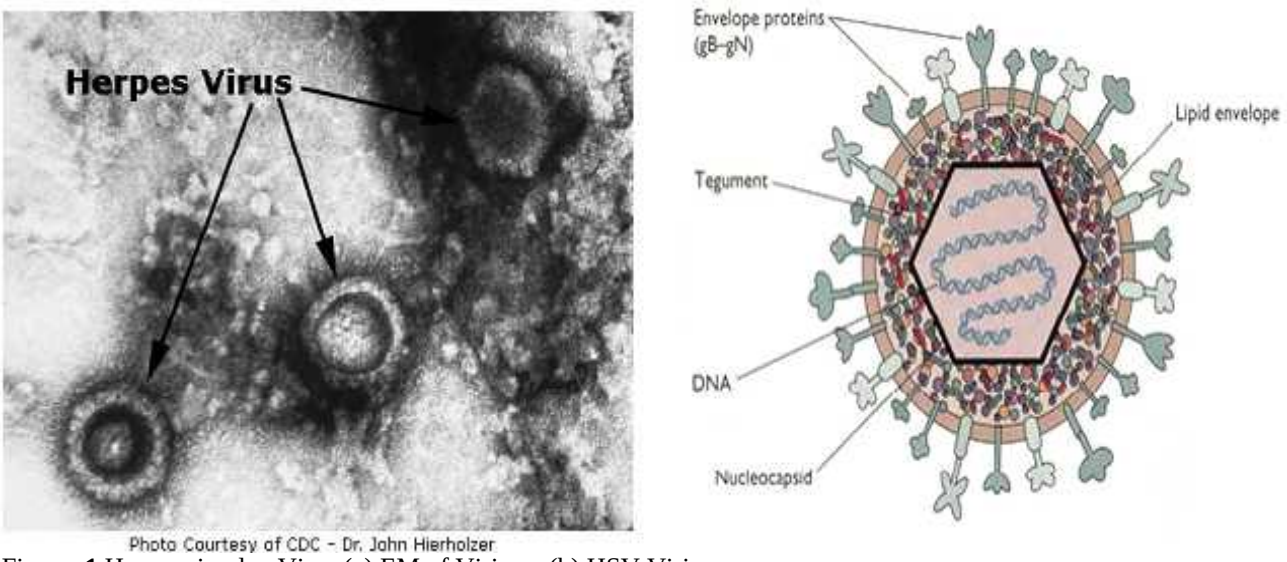

Figure**. 1** Herpes simplex Virus (a) EM of Virions, (b) HSV Virion.

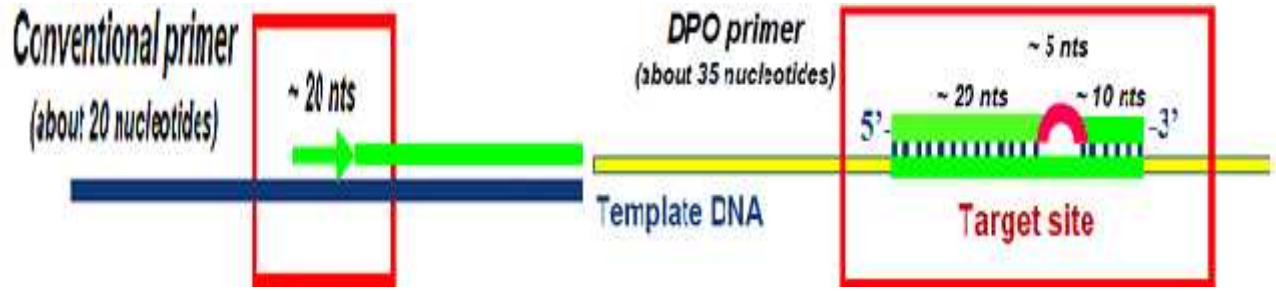

Figure. 2. Basic difference between conventional and DPO primers (Photo courtesy Seegene)

produce highly sensitive and specific diagnoses but they are pain full techniques with their non invasive, and time constraints discourage their regular use in clinical practice.

The Polymerase Chain Reaction (PCR) DNA amplification technique with useful addition of molecular biochemical is highly sensitive and specific for detection of HSV infections [10]. Seegene holds a new concept oligonucleotide technology, "Dual Priming Oligonucleotide (DPO™) technology, which provides freedom in primer design and PCR optimization and maximizes PCR specificity and sensitivity by fundamentally blocking non-specific priming at annealing step. The Seeplex® HSV2 ACE detection is based on three major processes: nucleic acid isolation; PCR amplification of target DNA using DPO™ primers; detection on automatic capillary electrophoresis system or agarose gel electrophoresis[10].

MATERIALS AND METHODS

Present study was done on Cerebrospinal fluid (CSF) specimens and genital secretion from SMI hospital and were transported at 4°C to Molecular Research Laboratory for further processing. Specimen were further processed for DNA extraction and subsequent amplification for HSv1 and 2 genotyping by DPO technology.DPO technology is a new addition to the Nucleic acid amplification techniques (NAAT) which provides freedom in primer designing, PCR optimization and maximizes PCR specificity and sensitivity by fundamentally blocking non-specific sequences (as depicted in figure 2 and 3). The assay includes internal control for the validation of the protocol which is introduced into every amplification reaction and is co-amplified with target DNA from the clinical specimen. Major drawback of PCR technique is false positive due to amplicons generation. Amplicon contaminations can be prevented by using 8-methoxypsoralen (8-MOP) which will extinguish the template activity of contaminated DNA thus preventing false positive. For setting up the PCR reaction, add 4 µl 5X HSV2 ACE PM (Primer Mixture primer pairs for HPS 1 &2 primer pair for internal control template of Internal control); 3 µl of 8-mop solution, 10 µl of 2X multiplex master mix (DNA polymerase, Buffer containing dNTPs {dATP, dCTP, dGTP, dTTP} $MgCl_2$ and stabilizers) with final addition of 3 µl of HSV DNA template into 0.2 ml of PCR tube. Cycling conditions for the amplification includes initial denaturation at 94°C for 15min, 30 cycles of denaturation at 94 °C for 0.5 minutes , annealing at 63°C for 1.5min and extension at 72°C for 1.5min. Give final extension at 72°C for 10 minutes followed by storage of amplicons at 4°C. After amplification, run the amplicons on 1.6% agarose gel by electrophoresis at 150 volts for 20 minutes. View the gel under U.V. transilluminator for interpreting the results. Amplicons size for Internal

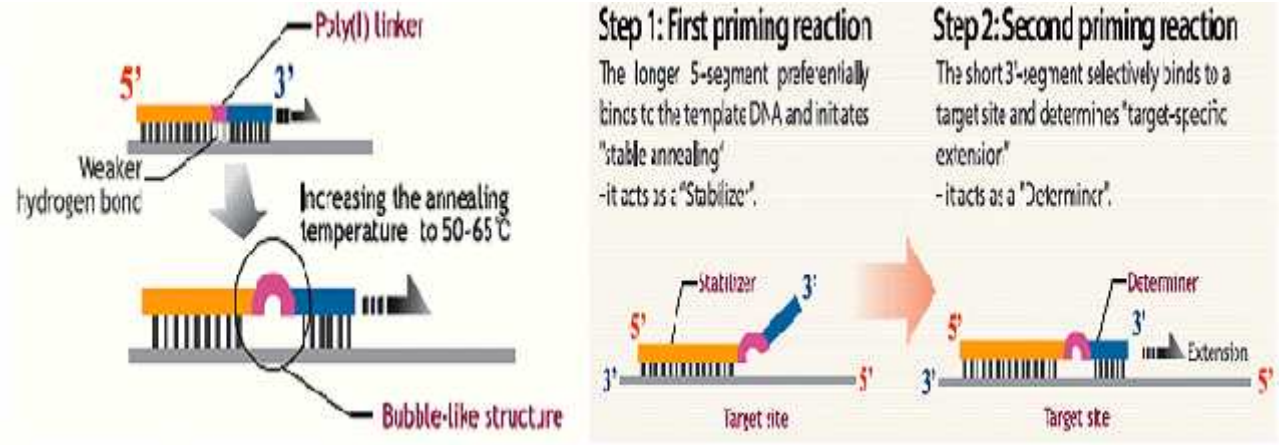

Figure. 3**.** Dual priming oligonucleotide PCR mechanism (Photo courtesy Seegene)

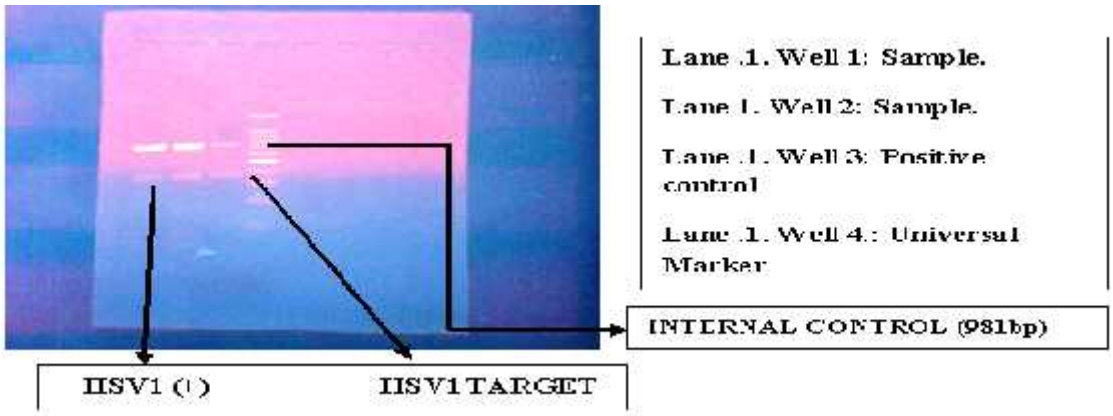

Figure 4: Gel picture depicting HSV genotyping positive results by DPO technology.

control at 981bp, for HSV-2 at 473 bp , HSV-1 at 300bp will be observed (Figure 4 and 5.).

RESULTS

Out of eight specimens amplified,two came positive for HSV.it was seen that when amplicons examined in 1.6% agarose gel,300 bp for HSV1 along with internal control was amplified, confirming type 1HSV,where as the amplicon size of 473 bp along with internal control confirmed HSV 2 type infection.

DISCUSSION

Human herpes viruses 1 and 2 (HSV-1 and HSV-2) are among the most common human viral pathogens. Various epidemiological factors determine the infecting HSV type with significant changes in the epidemiology of genital herpes and HSV-1 infections especially in developed countries. Following transmission of HSV to a susceptible person, symptomatic or asymptomatic infection may result with localized disease, the most common manifestation. HSV then establishes latency in the dorsal root ganglia which enables subsequent reactivation. The risk and rate of reactivation are dependent on numerous host factors and the infecting viral subtype. The number of diagnostic tests has increased with molecular methods improving our understanding of asymptomatic viral shedding. Antiviral therapy ameliorates the clinical manifestations and improves survival in neurological disease. Prophylactic antiviral strategies have been formulated for recurrent disease, but vaccine trials do not yet show clinically relevant benefit. A definitive diagnosis of HSV can be made by scraping a lesion with a Dacron swab and obtaining an FA and culture on the sample. Both FA and culture can distinguish HSV-1 from HSV-2. Because HSV is an intracellular virus, the diagnostic yield increases if the base of the lesion is scraped and an adequate number of cells are obtained. The use of a Dacron swab is preferred. The overall yield on lesions decreases as the lesion heals. Approximately 90% of positive HSV cultures will show growth by day 5. Recent studies have shown that HSV PCR of genital lesions has a significantly greater sensitivity than viral culture. The use of a Tzanck preparation to detect cytologic changes consistent with a herpes virus infection provides low sensitivity and specificity and thus is not recommended. Serologic testing for HSV, using a type-specific glycoprotein G (IgG)-based assay, can provide useful information in persons presenting with new lesions and no prior history of HSV infection; in addition, this assay distinguishes HSV-1 from HSV-2 antibody. Some experts

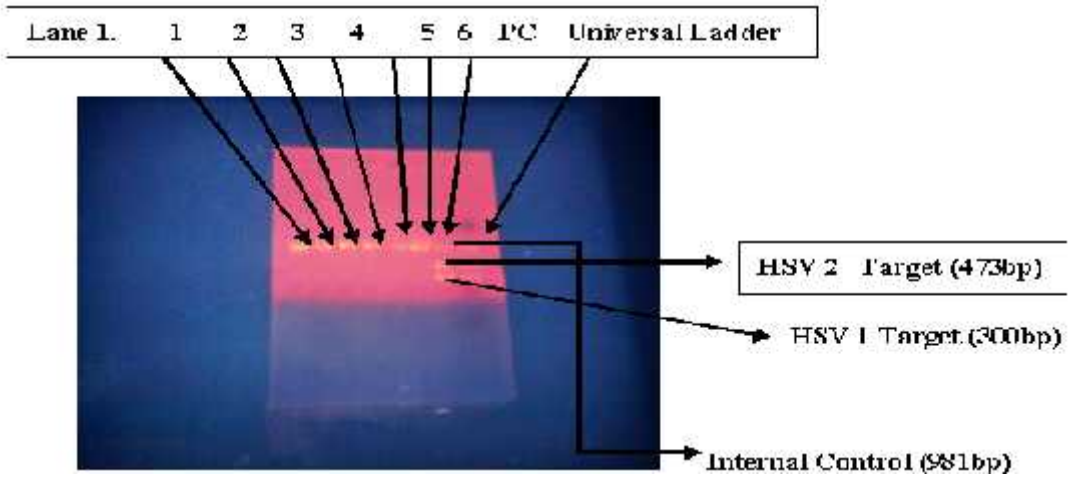

Figure 5: Gel picture depicting HSV genotyping Negative results by DPO technology.

now recommend performing HSV serologic testing in HIV-infected persons as part of the initial evaluation to help identify unrecognized or asymptomatic HSV-2 infection. Only 02 case positive out of 08 cases studied

CONCLUSION

Although HIV-infected persons infected with HSV can develop an array of manifestations, most patients remain asymptomatic. Among HIV-infected persons with HSV-2 infection, nearly all shed HSV-2 in the genital tract. Symptomatic HSV disease, when it occurs, most often manifests as orolabial or genital lesions, with HSV-1 typically isolated from orolabial lesions and HSV-2 from genital lesion. Patients with orolabial or genital HSV infection often have a sensory prodrome that precedes the onset of visible lesions. Once a visible lesion appears, it can evolve through several stages that may include vesicles, ulcers, and crusts. Among HIV-infected persons who do not have severe immune suppression, the clinical presentation of orolabial and genital HSV is usually similar to that seen in persons not infected with HIV. Patients with advanced immunosuppression, however, can develop chronic, extensive, deep ulcerated lesions. These chronic ulcerative lesions can appear anywhere on the body. Several reports have described patients who developed atypical ulcerative genital HSV lesions after starting highly active antiretroviral therapy, presumably caused by immune reconstitution; these lesions may be difficult to treat, despite the absence of acyclovir resistance.In this study, cervical and genital samples were analyzed using the DPO technique. Only two cases were positive out of 08 cases.

Herpes simplex virus (HSV) encephalitis is a serious infection but diagnosis previously required brain biopsy in certain cases due to the low sensitivity of cerebrospinal fluid (CSF) culture and serology. PCR now allows the detection of HSV DNA from CSF with 95% sensitivity thus avoiding invasive brain biopsy. Viral meningitis, commonly caused by either enteroviruses or HSV is more reliably detected by PCR when compared to culture and in a shorter time (one versus up to five days). HSV PCR can be multiplexed with other pathogens responsible for meningitis. Genital ulceration due to HSV, usually due to HSV type 2 infection is now routinely detected by PCR in many clinical microbiology laboratories due to its increased sensitivity over viral culture.

ACKNOWLEDGMENT

The authors are grateful to Honorable Chairman, Shri Guru Ram Rai Education Mission for his kind support and guidance.

Conflict of Interest: None

REFERENCES

1. World Health Organization. Guidelines for the management of sexually transmitted infections, Revised version.WHO/RHI/01.10 2003. Geneva: WHO, 2003.
2. World Health Organization. Guidelines for the management of sexually transmitted infections, Revised version.WHO/RHI/01.10 2003. Geneva: WHO, 2003.
3. Stewart JA, Reef SE, Pellett PE, et al. Herpesvirus infections in persons infected with human immunodeficiency virus. *Clin Infect Dis*;21(suppl 1):S114–20;1995.
4. Nahmias AJ, and Roizman BR: Infection with Herpes Simplex viruses 1 and 2. New Eng. J. Med. 289:667, 719, 781, 1983.
5. Nahmais AJ, Dannenbarger J, Wickliffe C, and Muther J: Clinical aspects of infection with Herpes simplex viruses I and II. In: AJ Nahmias, WR Dowdle, and RF Schinzai, eds., The Human Herpes Viruses, an Interdisciplinary Perspective, Elsevier/North-Holland Publishing co., New York, pp 2, 1980.
6. Ashley, RL: Current Concepts in Laboratory Diagnosis of Herpes Simplex Infections, In: SL Sachs, SE Strauss, RJ Whitney, and PD Griffiths, eds., Clinical Management of Herpes Virus, pp 139,1995.

7. Brown TJ, Yen-Moore A, Tyring SK. An overview of sexually transmitted diseases. Part I. J *Am Acad Dermatol*;41:511–32;1999
8. Munday, P.E., J. Vuddamalay, M.J. Slomka, and D.W.G. Brown. 1998. Role of type specific herpes simplex serology in the diagnosis and managemanet of genital herpes. Sex.Trasm.Infect. 74: 175-178;1998.
9. Centers for Disease Control and Prevention.Sexually transmitted diseases treatment guidelines 2002. MMWR 2002:51 (No.RR-6
10. Shin, So Youn, et al. "Evaluation of the Seeplex® Meningitis ACE Detection Kit for the Detection of 12 Common Bacterial and Viral Pathogens of Acute Meningitis." *Annals of laboratory medicine* 32.1 (2012): 44-49.