Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol.14 , Issue 1s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Comparative Evaluation Of Conventional Pcr Assays For The Detection Of Herpes Simplex Virus In Clinical Samples

Anjaria Mihir Bharatkumar[1], Dr. Sailesh Kumar[2]

[1] Department of Microbiology, Sunrise University, Alwar, Rajasthan, India.

[2] Department of Microbiology, Sunrise University, Alwar, Rajasthan, India.

Designation: Associate Professor...

Cite this paper as: Anjaria Mihir Bharatkumar, Dr. Sailesh Kumar (2025) Comparative Evaluation Of Conventional Pcr Assays For The Detection Of Herpes Simplex Virus In Clinical Samples . Journal of Neonatal Surgery, 14, (1s) 1574-1579

ABSTRACT

"Polymerase chain reaction (PCR) is an important tool for the diagnosis of herpes simplex virus (HSV) infections, especially in cerebrospinal fluid (CSF) samples. The study evaluated the performance of the in -house PCR assays that targets the UL30 genes for the general HSV detection and the US3 genes to separate HSV -1 and HSV -2. The results were compared with a commercial PCR kit. In-house assays continuously detected HSV-1", with no HSV-2 recognized, and comparable accuracy. Additionally, they provide a cost -effective and efficient option for regular diagnosis.....

Keywords: Cerebrospinal Fluid, clinical virology, molecular diagnostics, UL30 gene, US3 gene.

INTRODUCTION

The HSV is a neurotropic pathogen responsible for CNS infections, including encephalitis in infants and adults (Clanschimidt and Democators and Guilden, 2001). Early and accurate detection for timely antiviral treatment is important to reduce the risk of severe neurological complications and mortality.

PCR has greatly enhanced HSV diagnosis by enabling highly sensitive and specific detection of viral DNA in CSF, improved clinical accuracy and patient management (Gaudreu et al., 1998; 1998; Tabas et al., 1998).However, conventional PCR has certain limitations, including longer processing times and the need for post-amplification analysis, which increases the risk of contamination.

This study evaluates conventional PCR protocols using existing and newly designed primers for HSV detection, comparing their performance with a commercial kit to assess diagnostic efficiency, sensitivity, and specificity in clinical samples.

METHODOLOGY

Study Site & Subjects: Conducted at CIIMS, Nagpur, involving hospitalized patients with suspected HSE, including verified HSE cases (n=140) diagnosed via clinical/MRI findings and PCR, and non-HSE cases (n=179) comprising infectious (n=87) and non-infectious conditions. CSF samples were collected via lumbar puncture for PCR and microbiological testing.

DNA Extraction: Performed on 200 µl CSF using ZR Viral DNA Kit (Zymo Research, USA); stored at -20°C.

Primer Design: Created using Primer-BLAST; checked with NetPrimer & NCBI BLAST; synthesized by Sigma-Genosys.

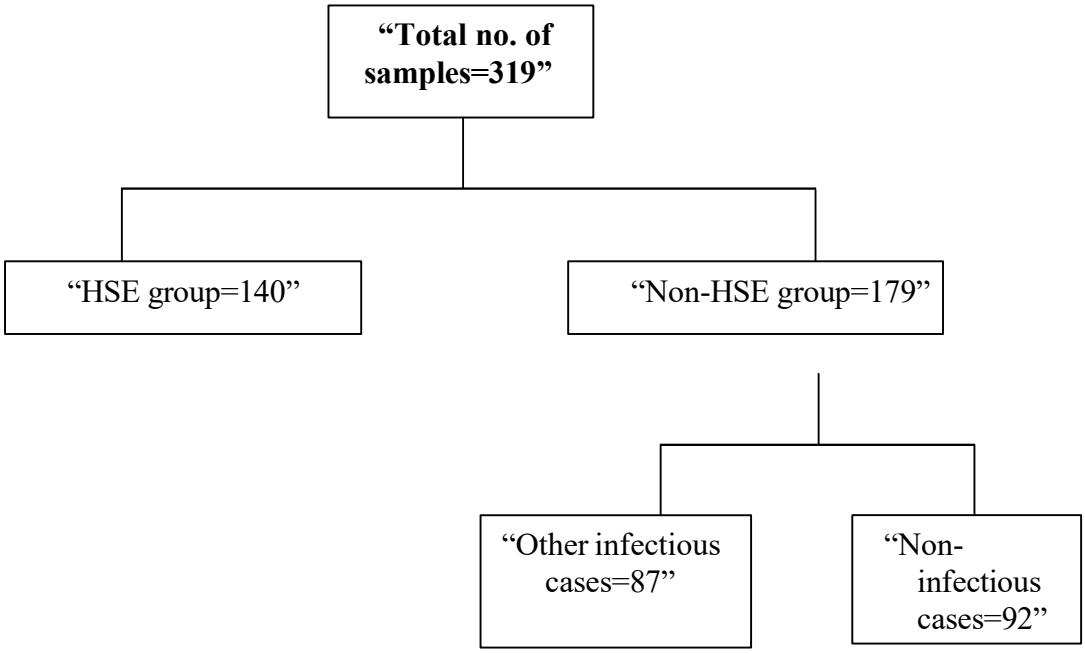
PCR Protocol: Used commercial HSV-1/2 kit (Cinnagen, Iran); 25 µl reactions with Taq polymerase, PCR mix, and 5 µl DNA.

Target Genes & Cycling Conditions: UL30 gene (179 bp) and US3 gene for HSV-1 (127 bp) and HSV-2 (144 bp); "PCR cycling included denaturation at 94°C, annealing at 65°C or 58°C, and extension at 72°C."

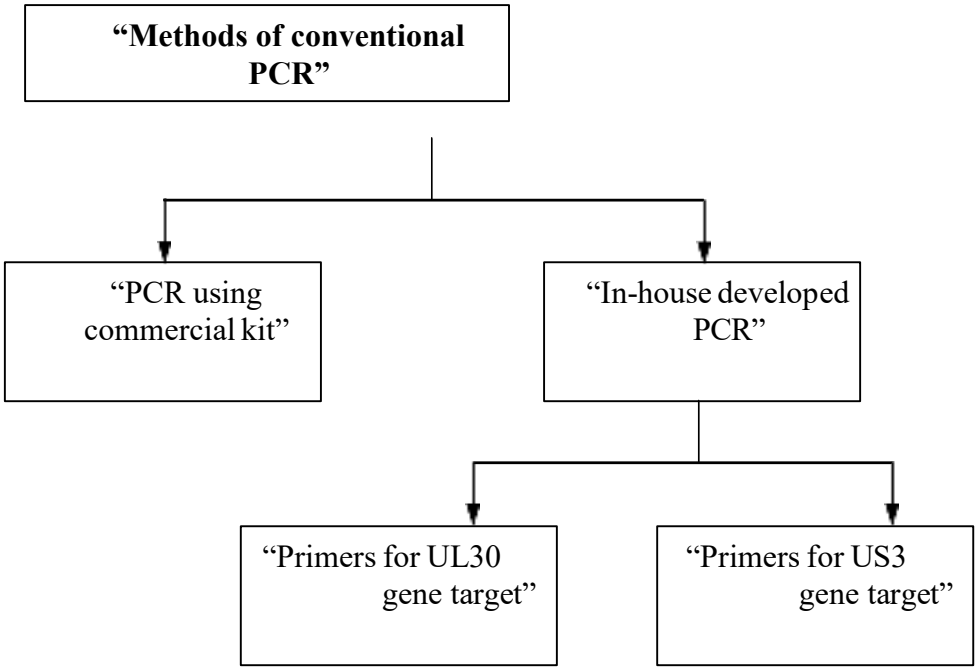
Analysis & Statistics: The the company Bio-R system was used for visualizing PCR results assessed by agarose gel chromatography; the Chi-square test for significance was used for analyzing the statistical data. (MedCalc v10.1.2).

Results

"Flow chart for classification of patients in different groups"



"Flow chart for analysis of samples of HSE and non-HSE using conventional PCR assays."



The UL30 assay detected HSV-1/2 commonly, while the US3 assay differentiated them. No cross-reactivity was found, and both assays showed high sensitivity with a 10 fg detection limit.

Table 1: "Detection of HSV DNA from CSF specimens"

Duration	"No. of specimens tested"	"No. of positive specimens by commercial kit"	"No. of positive specimens by in-house PCR"		
			"DNA Pol gene target"	"US3 gene target"	
				HSV - 1	HSV - 2
Sep 2020-Aug 2021	75	13	12	11	0
Sep 2021-Aug 2022	59	4	4	5	0
Sep 2021-Aug 2022	62	7	6	7	0
Sep 2022-Aug 2023	63	4	5	4	0
Sep 2023-Aug 2024	60	8	8	8	0
Total	**319**	**36**	**35**	**35**	**0**

Table 1 presents among 319 CSF samples, 36 tested positive for HSV using a commercial PCR kit, while 35 were positive with UL30 gene primers. Both assays identified 105 suspected HSE cases as negative. All 179 non-HSE cases tested negative for HSV, with no HSV-2 detected. The 35 UL30-positive cases were confirmed as HSV-1 positive. No significant difference was observed between the in-house and commercial PCR assays.

Table 2: "Male and female prevalence in total number of subjects and in patients with HSV positive CSF specimens by in-house PCR protocol"

"No. of total Subjects"		**"No. of Total Male / female ratio"**		**subjects**	**"HSV-positive male/female ratio"**
Male	**Female**				
43	31	1.42	8	4	2.00
31	28	1.07	2	2	1.00
42	20	2.15	3	4	0.75
39	26	1.46	2	2	1.00
37	22	1.68	5	3	1.67
192	**127**	**1.51**	**20**	**15**	**1.33**

Of the 319 CSF samples analysed, 192 (60%) were from male patients, and 127 (40%) were from female patients. Among the 35 HSV DNA-positive cases, 20 (57%) were male, and 15 (43%) were female. The male-to-female ratio of HSV-positive cases remained relatively consistent throughout the study (Table 2).

Table 3: "Age distribution in total number of suspected HSE subjects and in patients with HSV positive CSF specimens"

Age	Total subjects	No. of subjects positive
(years)	n (%)	n (%)
<10	17 (5.32%)	03 (8.57%)
10-20	35 (12.22%)	04 (11.42%)
21-30	52 (16.30%)	02 (5.71%)
31-40	49 (15.36%)	02 (8.57%)
41-50	38 (11.91%)	07 (20%)
51-60	67 (21.31%)	11 (31.42%)
>60	56 (17.55%)	04 (14.28%)

Table 3 shows that PCR-positive cases were most frequent in the 41–50 (20%) and 51–60 (31.42%) age groups. Clinical and laboratory findings did not significantly differ between PCR-positive and negative groups. Reduced consciousness was more common in PCR-positive patients (P=0.0967), though neurological deficits were not significant. CSF findings were similar, except for higher polymorphs in PCR-positive cases. CT/MRI abnormalities were more frequent in PCR-positive cases but not statistically significant. Post-treatment, PCR-positive patients showed varying degrees of impairment, while PCR-negative patients had a higher mortality rate (Table 4).

Table: 4: "Assessment of clinical symptoms in the confirmed HSE and suspected HSE group"

		Confirmed HSE (n=35)	Suspected HSE (n=105)	P value
Clinical n (%)	Motor deficit	20 (57.14%)	35 (33.33%)	0.2059
	Sensory abnormality Cranial nerve deficit	13 (37.14%)	29 (27.61%)	0.3939
	Reduced level of consciousness	22 (62.85%)	51 (48.57%)	0.2044
	Seizures	23 (65.71%)	50 (47.61%)	0.0967
		17 (48.57%)	46 (43. 81%)	0.7687
Imaging	"CT/MRI lesions n (%)"	19 (54.28%)	37 (35.23%)	0.0729
	"TLC"	0-500	0-500	
	"Lymphocyte (%)"	0-100	0-100	
	"Polymorphs (%)"	1-2	0-50	
	"Sugar (mg/dl)"	32.48-118.2	26-128	
CSF tests	"Protein (mg/dl)"	23.44-187.46	20.64-301.4	--

	"AFB negative"	35 (100%)	105 (100%)	
	"Gram stain negative"	35 (100%)	105 (100%)	
	"Bacterial culture negative"	35 (100%)	105 (100%)	
	"HIV negative"	35 (100%)	105 (100%)	
	"Normal"	12 (34.28%)	37 (35.23%)	
Clinical Outcome	Mild to moderate impairment	16 (45.71%)	34 (32.38%)	0.0879
	Severe impairment	5 (14.28%)	3 (2.85%)	
Death		2 (5.71%)	12 (11.43%)	

Discussion

PCR has transformed HSE diagnosis by detecting viral DNA in CSF (Baringer, 2000). This study analysed CSF samples submitted for HSV PCR (2008–2013) to optimize diagnostic conditions. Among 140 suspected HSE cases, 36 tested positives using a commercial PCR kit, while 35 were positive with UL30 gene primers. In-house PCR confirmed HSV-1 in all UL30-positive cases, with no HSV-2 detected.

HSE primarily affects middle-aged individuals (Koskiniemi et al., 1996), with most PCR-positive cases in the 41–50 (20%) and 51–60 (31.42%) age groups. No gender differences were observed (Riera-Mestre et al., 2009). PCR-positive cases showed higher associations with reduced consciousness (Domingues et al., 1998) but no significant CSF profile differences (Hanson et al., 2007). Clinical outcomes depended on age and consciousness at treatment initiation (Whitley et al., 1986). Prognosis is influenced by multiple factors (Raschilas et al., 2002). Commercial and in-house PCR assays demonstrated comparable efficacy for HSV CNS diagnosis.

Conclusion

The study successfully developed and evaluated conventional PCR-based protocols for HSV detection using both existing and newly designed primers. The performance of these in-house assays was compared with a commercially available PCR kit. The UL30 gene-targeting primers effectively identified HSV-1 in suspected HSE cases, with results comparable to the commercial kit. No cases of HSV-2 were detected in the study population. Additionally, PCR-positive cases were more frequently associated with reduced consciousness and CT/MRI abnormalities, though not statistically significant. Overall, the in-house PCR assays demonstrated high reliability and sensitivity, which offers a cost-effective option for regular HSV diagnosis in clinical settings

REFERENCES

1. Kleinschmidt-DeMasters, B.K. and Gilden, D.H. The expanding spectrum of herpesvirus infections of the nervous system. Brain Pathology 2001; 11(4):440-451.

2. Tebas P, Nease R F, Storch G A. Use of polymerase chain reaction in the diagnosis of herpes simplex encephalitis: a decision analysis model. Am J Med. 1998; 105:287–295.

3. Gaudreau A, Hill E, Balfour H H, Jr, Erice A, Boivin G. Phenotypic and genotypic characterization of acyclovir-resistant herpes simplex viruses from immunocompromised patients. J Infect Dis. 1998;178:297–303. doi: 10.1086/515626.

4. Baringer, J. R., and Pisani, P. (1994). Herpes simplex virus genomes in human nervous system tissue analyzed by polymerase chain reaction. Ann. Neurol. 36, 823–829.

5. Koskiniemi, M., Vaheri, A., & Taskinen, E. Cerebrospinal fluid alterations in herpes simplex virus encephalitis. Reviews of infectious diseases, 1984; 6(5), 608-618.

6. Riera-Mestre, A., Gubieras, L., Martínez-Yelamos, S., Cabellos, C., & Fernández-Viladrich, P. Adult herpes simplex encephalitis: fifteen years' experience. Enfermedades infecciosas y microbiología clínica, 2009; 27(3), 143-147.

7. Domingues R B, Tsanaclis A M, Pannuti C S, Mayo M S, Lakeman F D. Evaluation of the range of clinical presentations of herpes simplex encephalitis by using polymerase chain reaction assay of cerebrospinal fluid samples. Clin Infect Dis. 1997; 25:86–91.

8. Hanson, K.E., Alexander, B.D., Woods, C., Petti, C. and Reller, L.B. Validation of laboratory screening criteria for herpes simplex virus testing of cerebrospinal fluid. Journal of Clinical Microbiology 2007; 45(3):721-724.

9. Whitley, Richard J., Charles A. Alford, Martin S. Hirsch, Robert T. Schooley, James P. Luby, Fred Y. Aoki, Daniel Hanley, Andre J. Nahmias, Seng-Jaw Soong, and NIAID Collaborative Antiviral Study Group*. "Vidarabine versus acyclovir therapy in herpes simplex encephalitis." New England Journal of Medicine 1986; 314(3):144-149.

10. Raschilas, F., Wolff, M., Delatour, F., Chaffaut, C., De Broucker, T., Chevret, S., Lebon, P., Canton, P. and Rozenberg, F., 2002. Outcome of and prognostic factors for herpes simplex encephalitis in adult patients: results of a multicenter study. Clinical infectious diseases, 2002;35(3):254-260.

.