Diagnosis of Herpes Simplex Virus1 and 2 in Clinical Specimens by Tissue Culture and Polymerase Chain Reaction

Thesis

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Presented by

Eman Ibrahim Aly Mahmoud

M.B.B.CH, Faculty of Medicine, Ain Shams University

Under Supervision of

Prof. Aly Mohamed Zaki

Professor of Medical Microbiology and Immunology,
Faculty of Medicine, Ain Shams University

Dr. Walaa Shawky El-Sayed Khater

Assistant Professor of Medical Microbiology and Immunology
Faculty of Medicine, Ain Shams University

Dr. Marwa Kamal Assaad

Lecturer of Dermatology
Faculty of Medicine, Ain Shams University

Faculty of Medicine
Ain Shams University
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Dedication

This work is dedicated to ... ***my beloved parents****, for their support and encouragement,* ***my husband*** *for his support, continuous encouragement from step to other higher step and helping me to overcome the difficulties and* ***my lovely children*** *for being the light of my life.*

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List of Abbreviations

- Acyclovir (**ACV**)
- Ain Shams University hospitals (**ASUH**)
- Cell-mediated immune response (**CMIR**)
- Central nervous system (**CNS**)
- Cerebrospinal fluid (**CSF**)
- Cytomegalovirus (**CMV**)
- Cytopathic effect (**CPE**)
- Direct fluorescent antibody (**DFA**)
- Double-stranded DNA (**dsDNA**)
- Enzyme linked immunosorbant assay (**ELISA**)
- Enzyme Linked Virus Inducible System (**ELVIS**)
- Epstein-Barr virus (**EBV**)
- Food and Drug Administration (**FDA**)
- Glycoprotein G (**gG**)
- Herpes simplex encephalitis (**HSE**)
- Herpes simplex virus (**HSV**)
- Host cell factor 1 (**HCF1**)
- Human alveolar adenocarcinoma cell line (**A549**)
- Human herpesvirus (**HHV**)
- Iscove's Modified Dulbecco's Medium (**IMDM**)
- Latency-associated transcripts (**LAT**)
- Medical Research Council -5 (**MRC**-5)
- Negative predictive value (**NPV**)
- Normal african green monkey kidney fibroblast cells (*Cercopithecus aethiops*) (**CV-1**)

- Nucleic acid amplification tests (**NAATs**)
- Octamer - binding protein 1 (**Oct-1**)
- Open reading frames (**ORFs**)
- Origin of replication (**Ori-L**)
- Polymerase chain reaction (**PCR**)
- Positive predictive value (**PPV**)
- Primary herpetic gingivostomatitis (**PHGS**)
- Skin, eyes, and/or mouth (**SEM disease**)
- Standard deviation (**SD**)
- Trigeminal ganglion (**TG**)
- United Kingdom (**UK**)
- United States (**US**)
- Varicella-zoster virus (**VZV**)
- Vero and Human epithelial type 2 (**HEp-2**) cells
- Western blot (**WB**)

INTRODUCTION

Herpes simplex virus 1 (HSV-1) and 2 (HSV-2) are both members of the *Herpesviridae* family ***(Steiner et al., 2007).*** They cause a wide spectrum of clinical manifestations ranging from mucocutaneous oral and genital lesions to serious central nervous system (CNS) manifestations ***(Anderson et al., 2014).*** HSVs can remain latent following primary infection in the dorsal root ganglia and may reactivate in situations when the immune status is compromised causing life threatening conditions ***(Steiner and Benninger, 2013).***

Infections with HSVs are relatively common worldwide and it is believed to be even more common in developing countries more than developed ones ***(Anderson et al., 2014).*** HSV-1 is responsible for over 10% of all encephalitis cases and is considered to be the commonest cause of fatal sporadic viral encephalitis worldwide ***(Binnicker et al., 2014)***. Failure to reach diagnosis and start prompt antiviral therapy usually result in elevated mortality rates, lifelong neurologic sequels in survivors or disseminated disease as in case of neonatal infection with HSV-2. In these situations, the availability of rapid sensitive and specific diagnostic assays for HSVs are therefore crucial ***(Kimberlin, 2005***).

Various diagnostic methods have been described for the diagnosis of HSV infections including viral culture, direct antigen detection and molecular assays ***(Liu et al., 2015).***

Viral culture and isolation is considered the gold standard method, against which the performance of any other methods are tested ***(Slomka et al., 1998).*** Nevertheless, it had been criticized by being timely, laborious, needs highly skilled personnel, subjective, and results are affected by collection technique and transport conditions ***(Gitman et al., 2013).***

Some reports have shown that molecular assays are more sensitive, rapid than culture for the diagnosis of HSV in dermal and genital samples and they are considered the standard diagnostic assay for detecting herpes infection of the CNS as they are not only sensitive, rapid but also they are better alternative than brain biopsy ***(Liu et al., 2015).*** Yet, their relative high cost and inability to perform antiviral susceptibility testing are their main disadvantages ***(Strick and Wald, 2006).***

AIM OF THE WORK

This study aims to compare the performance to results of conventional polymerase chain reaction with those of cell culture in the detection of Herpes Simplex Viruses 1 and 2 in different clinical specimens.

HERPES VIRIDAE FAMILY

I. Historical Background

Herpes, from the ancient Greek means to creep or crawl ***(Whitley, 2001)***. Rome recognized herpes 2,000 years ago when the Roman Emperor Tiberius banned kissing because of the incidence of herpes ***(Glover, 1984)***.

In the 1830s, recurrent genital herpes was described and 60 years later, it was identified as a vocational disease of sexually transmitted infection. The virus itself was not identified until the 1950s. In 1971, it was proposed that two different types of herpes simplex virus (HSV) could cause infection ***(Gebreyohannes, 2014)***.

II. Taxonomy

The herpesviruses are double-stranded DNA (dsDNA) viruses belonging to Herpesvirales order. More than 150 individual viruses have been discovered and described in almost all species of vertebrates and invertebrates ***(Smith and Whitley, 2017)***.

Genetic analysis divides the Herpesvirales order into three distinct families ***(Davison, 2009)***. The family containing the human herpesviruses, Herpesviridae, is further divided into three subfamilies – Alphaherpesviridae,