Evaluation of Argene's Research Use Only, Real-Time PCR Reagents for HSV1 and HSV2 R-GENE

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Abstract

The Herpesviridae are a family of DNA viruses which are responsible for a wide spectrum of infections in humans. There are eight human Herpesviridae, of which Herpes simplex virus types 1 (HSV-1) and 2 (HSV-2) and Varicella Zoster Virus (VZV) are the most common in immunocompetent patients. Usually benign, the infections linked with these viruses can nevertheless develop severe clinical forms such as encephalitis, meningitis, retinitis and neonatal infections. Various antivirals have proved their worth in efficiently treating these pathologies if prescribed early. Among the severe forms of HSV-1, HSV-2 or VZV infections in adults, is HSV-1-induced encephalitis. At present, there is no commercially available FDA-approved kit for the HSV-1 and 2 diagnosis by polymerase chain reaction (PCR) method. Molecular diagnostic laboratories must therefore develop and validate their own in-house assays. The Argene HSV1 HSV2 VZV R-gene™ kit, a real-time PCR assay, may provide a standardized solution to diagnose HSV1, HSV2, and VZV viral infection in clinical specimens. In this study we evaluated the Argene HSV1 HSV2 VZV R-gene™ kit only for HSV 1 & 2 from cerebrospinal fluid (CSF) specimens and compared results of this kit with results obtained from our in-house HSV 1 & 2 assay.

DNA from CSF samples (n = 60) were coded, extracted, and tested blindly in a real-time PCR assay using both methods (our in-house HSV 1 & 2 qualitative assay, Argene HSV1 HSV2 VZV R-gene™ quantification kit). Quantification of HSV1 and HSV2 viral load was performed using a standard curve of known HSV1 and 2 DNA concentrations, the Log results of each assay were plotted against those of the reference laboratory, and r values calculated.

A strong linear relationship was identified between our in-house assay and Argene HSV1 HSV2 VZV R-gene™ kit. In both assay examined, clinical concordance was 100% (clinical sensitivity and specificity were both 100%). The HSV1 HSV2 VZV R-gene™ k gave reproducible results and this assay significantly simplified detection of HSV 1 & viral infection compared to our in-house assay. As a result of employing Argene's HSV HSV2 VZV R-gene™ kit savings should be realized by eliminating much of the manu set-up required for in-house assays thereby improving turn-around time. In ou opinion, the HSV1 HSV2 VZV R-gene™ kit would be suitable for use in diagnost laboratories that do not have the facilities and staff to design and validate their own i house assays for the molecular diagnosis of HSV1 and HSV2 DNA.

Introduction and Aim

Herpes simplex virus types 1 (HSV-1) and 2 (HSV-2) are members of the Herpesviridae family of DNA viruses. The primary infection is limited to the mucous membranes and skin but the virus can persist in its host in a latent state and can be reactivated to give recurrent infections, particularly in immunocompromised patients. HSV-1 is the etiological agent in the most common form of sporadic, potentially fatal encephalitis in adults, while HSV-2 causes neonatal encephalitis in newborn babies, which can lead to serious neurological disorders. Antiviral medications can be effective treatments against HSV if prescribed early and at appropriate doses.

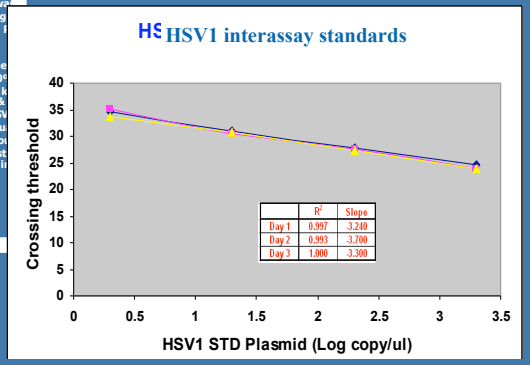
Although conventional immunological culture and detection techniques are suitable for diagnosing the benign primary infections caused by these viruses, they are unsuitable for severe infections of the central nervous system (CNS) and congenital infections. Detection of low viral titers often found in cerebrospinal fluid requires the high level of sensitivity that is provided by PCR-based assays. At present, there is no commercially available FDA-approved kit for PCR-based quantification of HSV-1 HSV-2 VZV viral load in the United States, and laboratories which engage in quantitative analysis of HSV must therefore develop and validate their own in-house assays. The Argene HSV-1 HSV-2 VZV R-gene™ kit enables the quantification of HSV-1 and HSV-2 in cerebrospinal fluid, gynecological smears, ears nose throat (ENT) and ophthalmologic specimens, cutaneous and mucous smears, and broncho-alveolar liquid (BAL).

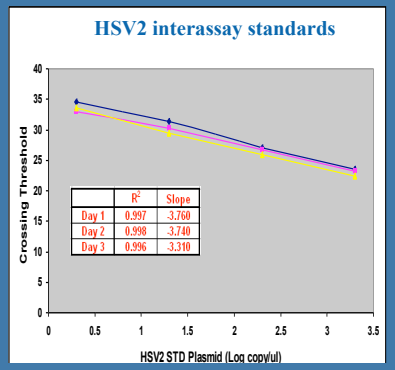
In this study we evaluated the Argene HSV-1 HSV-2 VZV R-gene™ kit and compared results of this kit with results obtained from our in-house HSV-1 and HSV-2 assays for validation.

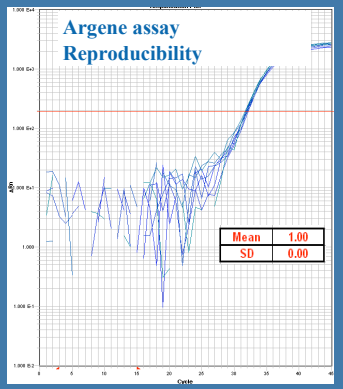
Materials & Methods

DNA from cerebrospinal fluid (CSF), (n = 60), obtained from our molecular diagnostics laboratory, was extracted using the NucliSens easyMAG automated extraction platform (Biomérieux). The samples were received coded and were tested blindly in a real-time PCR assay employing the LightCycler® 1.0 (Roche Diagnostics) or the ABI Prism® 7900HT (Applied Biosystems) using one of the following two assays: (1) Our in-house HSV 1 & 2 qualitative real-time PCR assay; or (2) Argene HSV1 HSV2 VZV R-gene™ quantification kit. Quantification of samples was performed using a standard curve of known HSV1 and 2 DNA concentrations. Log_{10} results of each assay were plotted against those of the in-house assay and R^2 values calculated.

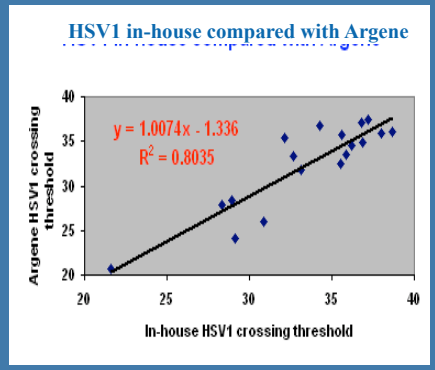
Results







In-house HSV1 & 2	Argene HSV1 & 2 Pos	Neg
Pos	19	20
Neg	41	40



Conclusions & Discussion

- A strong linear relationship was identified between our in-house assay and Argene's HSV1 HSV2 VZV R-gene™ kit.
- In every assay examined, clinical concordance with the in-house assay was 100% (clinical sensitivity and specificity were both 100%)
- The HSV1 HSV2 VZV R-gene™ kit assay was easy to perform and gave reproducible results.
- The HSV1 HSV2 VZV R-gene™ kit assay had the advantage of an internal control for assessment of extraction efficiency and lack of inhibitory factors for amplification.
- Savings should be realized with the institution of the HSV1 HSV2 VZV R-gene™ kit by:
  - Eliminating much of the manual set-up required for in-house assays
  - Decreased turn-around time
  - Increased number of samples per PCR run (32 samples per run with HSV1 HSV2 VZV R-gene™ kit v. 16 samples per run with in-house assay)
  - Less space required for storage of reagents (all reagents in one box)

In our opinion, the HSV1 HSV2 VZV R-gene™ kit would be suitable for use in diagnostic laboratories that do not have the facilities and/or staff to design, validate, and perform in-house assays.

References & Acknowledgements

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