Improvement in quantitative detection of Human Herpesvirus 6 with a ready-to-use PCR-based Real Time PCR assay

Sentinel CH. SpA, via Robert Koch 2, 20152 Milano, Italy

Abstract
Background: Human Herpesvirus 6 (HHV-6) is a set of two closely related herpes viruses known as HHV-6A and HHV-6B. HHV-6B infects nearly 100% of human beings, typically before the age of three. HHV-6A can promote the progression of HIV disease toward AIDS, as both clinical and experimental evidence suggest. Like other human herpesviruses, HHV-6 establishes latency in mononuclear cells. This virus infects virtually all humans. HHV-6A and HHV-6B, which share certain homology, but are clearly distinct, both virologically and at the time when other opportunistic infections usually appear. Both HHV-6 viruses are highly cell associated and can be detected in plasma briefly during the initial infection or acute reactivation. Detection of HHV-6 DNA in plasma generally means the patient has an active infection. The aim of this work was to evaluate the performance of a new assay designed to detect and quantify HHV-6 DNA in human samples.

Material and methods: The assay was developed as a ready-to-use test containing all the required elements for the amplification of both HHV-6 DNA fragment and human beta globin gene. The two sets of primers and probes are combined in a lyophilized and ready-to-use mix, co-amplified and detected by a Real-Time PCR instrument. In the present study, several samples obtained from a hospital were previously tested with "artus HHV-6 RG PCR Kit" (Qiagen), were investigated. PCR reactions were performed on nucleic acids extracted from plasma, whole blood and cerebrospinal fluid (CSF).

Results: The novel freeze-dried ready-to-use assay demonstrated robust and accurate target amplification, according to the data obtained at a hospital. The assay did not cross-react with any of the other Human herpesviruses tested. The test showed a LoD of 5 genome copies/reaction and a LoS of 2 genome copies/reaction. The panel of 33 samples were extracted at San Raffaele Hospital and previously tested with "artus HHV-6 RG PCR Kit" (Qiagen). The assay was developed as a ready-to-use test containing all the required elements for the amplification of both HHV-6 DNA fragment and human beta globin gene as internal control. The two sets of primers and probes are combined in a lyophilized and ready-to-use mix, co-amplified and detected by a Real-Time PCR instrument. The high-sensitivity and specificity of this assay, linearity and quantitation performances, associated with the ready-to-use and room temperature storage, would have a direct impact on the early and correct management of the affected patients.

Background: HHV-6 is a member of the β-herpesvirus subfamily of human herpesviruses. There are two subtypes of HHV-6, type A and type B, which share certain biological properties and a high level of sequence homology, but are clearly distinct, both virologically and epidemiologically. HHV-6 infects multiple cell lines and tissues and establishes latent infection in mononuclear cells. This virus infects virtually all children within the first three years of life and, like other herpesviruses, it establishes latency after primary infection. In immunocompromised patients who undergo transplantation, HHV-6 may exit the latent state and actively replicate, causing serious consequences from a clinical point of view. Real Time PCR assays are able to identify the presence of HHV6 virus in biological specimens and they allow to collect clinical indications about the efficacy of therapy, anti-viral drugs resistance, preventive treatments and disease progression.

Materials and Methods: This HHV6 detection assay is a freeze-dried and ready-to-use kit that includes all the required elements for the amplification of nucleic acid targets (Figure 1). Its intended use is the detection of HHV6 in human samples.

Figure 1: HHV6 assay components and freeze-dried mix. A specific set of primers and probe was designed to amplify a 118 bp DNA fragment within a conserved region of HHV6 genome, a second set of primers and probe amplifies another conserved region of 100 bp of HHV6 genome and a third set of primers and probe amplifies a 127 bp Human Beta Globin gene fragment, used as an internal amplification control (IC). In this assay the three targets are amplified and detected by a CFX96 Real-Time PCR instrument (BioRad) using the protocol indicated in Table A.

<table>
<thead>
<tr>
<th>Step</th>
<th>Cycle/Number</th>
<th>Denaturation</th>
<th>Annealing</th>
<th>Total run</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>95°C 2 min</td>
<td>-</td>
<td>95°C 2 min</td>
</tr>
<tr>
<td>2</td>
<td>45</td>
<td>95°C 15 sec</td>
<td>60°C 1 min</td>
<td>-</td>
</tr>
</tbody>
</table>

Table A: Thermal cycler protocol. HHV6 assay's performances were evaluated on:
- a total of 18 plasma, whole blood and CSF samples previously diagnosed as positives;
- a total of 15 plasma whole blood and CSF samples previously diagnosed as negatives;
- a quantified reference strain obtained from Virceel (Virceel – Granada, Spain).

Conclusions: Lyophilization is a widely applicable technology that can be used for stabilizing at room temperature different amplification mixes with a variety of applications. The presented study includes strong positives as well as weak positives and negatives. Obtained data totally agreed with HSR results and confirmed 18 positive samples and 15 negative samples. Even in presence of very low levels of infection, developed test was able to detect the presence of HHV6 in sample, with a high level of agreement of quantitation (CT) with the reference system.

Figure 3: Diagnostic sensitivity: Panel of 18 positive samples; human beta globin gene (IC, internal amplification control) amplification in blue; target genomic DNA in green.

Figure 4: Diagnostic specificity: Panel of 15 negative samples; human beta globin gene (IC, internal amplification control) amplification in blue; target genomic DNA in green.

The panel of 33 samples were extracted at San Raffaele Hospital (HSR) with QIAsymphony DNA extraction system (Qiagen) from human plasma, whole blood and CSF and previously diagnosed as positives and negatives with the "artus HHV-6 RG PCR Kit" (Qiagen) (Table B). The assay was developed as a ready-to-use test containing all the required elements for the amplification of both HHV-6 DNA fragment and human beta globin gene as internal control. The two sets of primers and probes are combined in a lyophilized and ready-to-use mix, co-amplified and detected by a Real-Time PCR instrument. The analytical sensitivity of the assay was evaluated with several dilutions of HHV6 strain. The genomic DNA of HHV6 was mixed with Human genomic DNA, simulating human samples (Figure 2). The Analytical sensitivity of the assay was evaluated with several dilutions of HHV6 strain. The genomic DNA of HHV6 was mixed with Human genomic DNA, simulating human samples (Figure 2).