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Short communication

Improved multiplex-PCR and microarray for herpesvirus detection from CSF

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Abstract

Background: A multiplex-PCR and microarray-based method was designed for detection of eight herpesviruses from clinical specimens.
Objectives: To improve the method, especially for detection of herpes simplex (HSV) and varicella-zoster viruses (VZV), and to update and validate the method using positive cerebrospinal fluid (CSF) samples.
Study design: A new primer pair for HSV-PCR and four detection oligonucleotides for HSVs were designed. Two new detection oligonucleotides for VZV and additional oligonucleotides for CMV, EBV, HHV-6 and -7 were designed. The new and previous multiplex-PCR and microarray method were tested in parallel with dilution series of commercial herpesvirus DNAs and 20 CSF specimens positive for HSV-1, HSV-2, or VZV.
Results: New method was more sensitive for detection of HSVs and both two new detection oligonucleotides for VZV functioned well at low levels of viral DNA.
Conclusions: The revised HSV-PCR and new HSV- and VZV-oligonucleotides were found to function well and be more sensitive, thereby increasing reliability of the method.

Keywords: Herpesviruses; Multiplex-PCR; Microarray; CNS

1. Introduction

Central nervous system (CNS) infections are caused by many viruses and bacteria, among which herpesviruses are very important viruses causes of encephalitis and meningitis. The reliable and sensitive detection of herpesvirus infection relies on nucleic acid detection and serological methods (Hanson et al., 2007). Optimal detection methods should accommodate some sequence variation as new strains appear (Dolan et al., 2004, 2006; Norberg et al., 2006; Tyler et al., 2007).

We sought to update previously published multiplex-PCR and microarray methods for simultaneous detection of herpesviruses (Jääskeläinen et al., 2006). A new multiplex-PCR for herpes simplex viruses (HSVs) and new detection oligonucleotides [U1] for other herpesviruses were designed and tested. A panel consisting of herpesvirus-positive cerebrospinal fluids (CSF) was used to compare the published and the updated methods.

2. Materials and methods

2.1. Controls and clinical specimens

DNA controls of HSV-1 strain MacIntyre, HSV-2 G, CMV AD169, EBV B95-8, HHV-7 H7-4, HHV-6A U1102, HHV-6B Z-29, and varicella-zoster virus (VZV) Rod (Advanced Biotechnologies, Columbia, MD) were used for dilution series.

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Twenty-two CSFs were collected from specimens tested with HSV-PCR (Piiparinen and Vaheri, 1991; Vesanen et al., 1996), and VZV-PCR (Echevarria et al., 1994; Koskiniemi et al., 1997) at HUSLAB (Helsinki, Finland). Ten CSFs were positive for HSV-1 or HSV-2, 10 for VZV, and two were negative. Extractions were performed with High Pure Viral Nucleic Acid Kit (Roche Diagnostics, Basel, Switzerland). From a 200 μl sample 50 μl of DNA solution was obtained.

2.2. Oligonucleotides and multiplex-PCR primers

A new primer pair for HSVs and detection oligonucleotides for HSVs (Table 1) were designed using Primer3 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi; Rozen and Skaletsky, 2000) and DNA mfold software (http://frontend.bioinfo.rpi.edu/applications/mfold/cgi-bin/dna-form1.cgi; Zuker, 2003). With the new HSV-primer pair a 249-bp amplicon was produced. Altogether, there were four new detection oligonucleotides for HSVs; two general and two for discrimination of HSV types. For VZV there were two detection oligonucleotides and for CMV, EBV, HHV-6 and -7 one new oligonucleotide (Table 2). The new and published primers were use to carry out multiplex-PCRs according to Jääskeläinen et al. (2006).

2.3. Microarrays

SAL-1-slides (Asper Biotech, Tartu, Estonia) were spotted using a microarrayer (OmniGrid®, GeneMachines, Huntingdon, UK), spotting solution (1× MSS, ArrayIt, TeleChem International Inc., Sunnyvale, CA), and SMP5-pin (TeleChem International Inc.). Spotting was performed at Biomedicum Genomics (University of Helsinki). There were 48 subarrays per slide and 70 spots per subarray consisting of 16 detection oligonucleotides for herpesviruses (Tables 1 and 2, and Jääskeläinen et al., 2006) and two unspecific oligonucleotides.

2.4. Microarray reactions and analyzing

Microarray reactions were performed according to Jääskeläinen et al. (2006) with few modifications. Two multiplex-PCRs were carried out and pooled before denaturing at 96 °C for 2 min. Denatured amplicons were transcribed into ssRNA using faster AmpliScribe™ T3 FLASH Transcription Kit (Epicentre, Madison, WI) at 42 °C for 1 h following the manufacturer's instructions. The washing time was decreased from 5 min to 3 min, and before final washing the microarrays were dipped in 50 mM NaOH. Microarrays were analyzed using a ScanArray™ Express scanner, ScanArray™ and QuantArray™ software (PerkinElmer, Wellesley, MA). The cut-off value was determined according to Jääskeläinen et al. (2006).

3. Results

3.1. Detection limits

The new multiplex-PCR and microarray method for HSVs provided a detection limit of 5 copies for HSV-1 and HSV-2. The detection limit for VZV was 7 copies with the new VZV-oligonucleotides. With the published VZV-oligonucleotide one of the duplicate tests remained negative and the other was barely positive for detecting 13 VZV copies.

Additional oligonucleotides for CMV and HHV-6, and EBV and HHV-7 gave good positive signals of 5–20 copies, and 40–60 copies with microarray, respectively. The detection limit was determined when >95% of parallel dilutions were positive. Microarray reactions with serially diluted samples were repeated a minimum of 10 times per control.

3.2. Clinical specimens

Twenty-two CSF specimens were tested with both published and newly designed methods in parallel to evaluate the improvement of the updated method for HSV and VZV detection. These experiments (Table 3) indicated that the newly designed multiplex-PCR and microarray were superior. In 9 out of 10 HSV-positive cases (90%) the updated method gave a positive result, while only 3/10 (30%) were positive with the published method. For the VZV panel the results were 10/10 (100%) and 5/10 (50%), respectively. Negative CSFs, water controls of extractions and multiplex-PCRs were negative.

4. Discussion

Sensitivity is critical for detecting of pathogens causing CNS infections. Among herpesviruses HSV and VZV are most often involved in CNS infections (Hanson et al., 2007). Rapid simultaneous detection of herpesviruses is relevant for proper drug treatment and beneficial for patient recovery. Microarray is an efficient method for detection of multiple targets from small volume samples. Stöcher et al. (2003) set up four real-time PCRs for simultaneous detection of five human herpesviruses in a single run. Their PCRs were sensitive but performed separately therefore increasing the amount of extraction needed for parallel testing.

When the published method for HSVs was studied more closely, the secondary structure of the HSV-PCR product was suspected of blocking efficient ssRNA production from amplicons, thereby decreasing the sensitivity of the subsequent hybridization step. A new multiplex-PCR for HSVs and oligonucleotides for herpesviruses were designed to increase the sensitivity for detecting HSV-1, -2 and VZV. New detection oligonucleotides for other herpesviruses (Table 2) were also designed to supplement the published oligonucleotides, increasing the potential for detecting diverse PCR amplicons.