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Multiplex-PCR and oligonucleotide microarray for detection of eight different herpesviruses from clinical specimens

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Abstract

Background: Human herpesviruses cause clinically important diseases, e.g. infections of the central nervous system. New diagnostic tools are required for rapid and reliable detection of these viruses.
Objectives: A microarray-based method was designed for detection of eight human herpesviruses in cerebrospinal fluid (CSF), whole blood, plasma, serum and proficiency-testing specimens.
Study design: Herpes simplex type 1 and 2, varicella-zoster, cytomegalo-, Epstein-Barr and human herpes viruses 6A, 6B and 7 were amplified from clinical specimens by two multiplex-PCRs and transcribed to single-stranded RNAs which were hybridized to oligonucleotides on microarray. The results were compared to those from conventional PCR. In total, 227 specimens were tested including 23 CSF, 10 whole blood, 73 plasma, 10 proficiency-testing samples and 111 negative control samples.
Results: Concordant results were obtained in 214/227 (94%). Microarray detected 10 possible double and one triple infection. Negative control samples (70 serum, 30 CSF and 11 proficiency-testing samples) were all negative.
Conclusions: Microarray is suitable for detection of multiple herpesviruses in clinical samples.

Keywords: Herpesvirus; Multiplex-PCR; Microarray

1. Introduction

Herpesviruses cause a number of human diseases of clinical importance. Especially diseases of the central nervous system (CNS) and complications in immunocompromised patients can result from infection or reactivation by herpes simplex type 1 or 2 (HSV-1, HSV-2), cytomegalovirus (CMV), Epstein-Barr virus (EBV), varicella-zoster virus (VZV) or human herpes virus 6 (HHV-6) (Aalto et al., 2003; Aurelius et al., 1991; Koskiniemi et al., 2002; Piiparinen et al., 2002). In addition to HHV-6, human herpes virus 7 (HHV-7) has been reported to cause exanthema subitum and documented to cause severe complications in transplant recipients (Suga et al., 1997; Tanaka et al., 1994). Virus isolation, nucleic acid detection and a variety of serological methods are currently used to detect herpesvirus infections (Chiu et al., 1998; Espy et al., 2000; Ihira et al., 2002; Pitkäranta et al., 2000; Read et al., 2001). Multiplex-PCR assays have been developed to meet the demand for fast detection of herpesviruses (Aberle and Puchhammer-Stockl, 2002; Druce et al., 2002; Hudnall et al., 2004; Read and Kurtz, 1999).

Microarray technology provides a promising new approach in diagnostic microbiology and several applications have been introduced, e.g. for detection and genotyping of influenza, and zoonotic, papilloma and respiratory tract viruses (Gemignani et al., 2004; Sengupta et al., 2003; Wang et al., 2002).

We sought to develop an efficient method for simultaneous detection and genotyping of several herpesviruses. Two multiplex-PCRs and a microarray were designed for eight

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Table 1
List of commercial viral DNA controls (Autogen Bioclear, Wiltshire, UK) and cell supernatants used for optimization and testing of multiplex-PCR and microarray

Virus	Strain	Source of sample
HSV-1	MacIntyre	Commercial viral DNA
HSV-2	G	Commercial viral DNA
CMV	AD169	Commercial viral DNA
EBV	B95-8	Commercial viral DNA
HHV-7	H7-4	Commercial viral DNA
HHV-6A	U1102	Commercial viral DNA
HHV-6B	Z-29	Commercial viral DNA
VZV	Rodstrain	Commercial viral DNA
HHV-6A	In-house control[a]; GS	Cell supernatant
HHV-6B	Z-29	Cell supernatant

[a] Clinical isolate used as an in-house control.

herpesviruses. Microarray-based detection was compared to current in-house diagnostic PCRs.

2. Materials and methods

2.1. Viral DNA and clinical specimens

Serial 10-fold dilutions of commercial viral DNA controls (started from one million copies or virus particles) and cell culture supernatants were prepared as standards (Table 1). In total, 106 clinical specimens were collected from pretested herpesvirus-positive samples from solid organ and bone marrow transplant patients, and patients with neurological symptoms, including plasma (73), whole blood (10), or cerebrospinal fluid (CSF) (23). Proficiency-testing samples (10) were derived from a VZV (4) and an HSV (6) proficiency-testing program 2004 (QCMD, Glasgow, Scotland, UK) (Table 2). To study the specificity of the microarray, we also tested negative control clinical samples (111), including 70 sera from patients with suspected epidemic nephropathy, 30 previously tested CSFs, and 11 samples (Table 2) from a 2004 enterovirus-proficiency program negative for herpesviruses DNA.

2.2. DNA extraction

DNA was extracted using MagNA Pure LC instrument and Total Nucleic Acid Kit (Roche Diagnostics, Basel, Switzerland), High Pure Viral Nucleic Acid Kit (Roche Diagnostics) or chloroform-phenol extraction depending on the protocol used in diagnostics.

2.3. Multiplex-PCR primers and oligonucleotides

The primer pair for HSV-1 and -2 (multiplex-PCR1) was modified from published primers (Piiparinen and Vaheri, 1991). Oligonucleotides and primers for CMV, EBV, VZV, HHV-6A, HHV-6B and HHV-7 (multiplex-PCR2) 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://www.bioinfo.rpi.edu/applications/mfold/old/dna/; Zuker, 2003). T3 RNA polymerase promoter sequence was included in the reverse multiplex-PCR primers (Tables 3 and 4).

2.4. Multiplex-PCR amplification

Multiplex-PCR1 was used to identify HSV-1 and HSV-2 resulting in a 264-bp amplicon. Multiplex-PCR2 contained primer pairs for amplification of CMV, EBV, VZV, HHV-6A, HHV-6B and HHV-7 resulting in 220, 273, 186, 179 bp (both HHV-6A and HHV-6B) and 230 bp amplicons, respectively. Both multiplex-PCRs were carried out for each sample.

The multiplex-PCR1 was carried out in a final volume of 50 μl containing 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 0.01% (w/v) gelatin, 0.2 mM dNTPmix (Finnzymes, Espoo, Finland), 0.6 μM of primers, 12.5 U AmpliTaq Gold polymerase (Applied Biosystems, Foster City, CA) and 2 mM $MgCl_2$. The multiplex-PCR2 was performed in a final volume of 53 μl containing 47.2 mM KCl, 9.4 mM Tris–HCl (pH 8.3), 0.009% (w/v) gelatin, 0.19 mM dNTPmix (Finnzymes), 0.56 μM of each primers, 12.5 U AmpliTaq Gold polymerase

Table 2
Virus proficiency programs, strains, viral DNA loads and panel formats of proficiency-testing samples

Virus proficiency program	Strain	Viral DNA load (Gequiv./ml)	Panel format
Varicella-zoster virus, 2004	VZV strain 9/84 SMI	700	Lyophilized cultured VZV
		70000	Lyophilized cultured VZV
		7000000	Lyophilized cultured VZV
	HSV-1 ATCC strain MacIntyre	210000000	Lyophilized cultured HSV
Herpes simplex virus, 2004	HSV-1 ATCC strain MacIntyre	24000	Freeze-dried inactivated samples
		1500	Freeze-dried inactivated samples
	HSV-2 ATCC strain MS	15000	Freeze-dried inactivated samples
		15000000	Freeze-dried inactivated samples
	VZV strain 9/84 SMI	500000	Freeze-dried inactivated samples
	Neg	–	Freeze-dried inactivated samples
Enterovirus, 2004[a]	Selected EV serotypes	–	Lyophilized cultured EV

Gequiv./ml, genome equivalent per ml; neg, negative; EV, enterovirus. All proficiency-testing samples were diluted in sterile water (1 ml).
[a] Eleven enteroviral proficiency-testing samples contained enteroviruses (8/11), rhinoviruses (1/11) and two were negative. Both herpesvirus proficiency programs contains HSV and VZV.