Multiplex detection of human herpesviruses from archival specimens

using MALDI-TOF mass spectrometry

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Abstract

The human herpesviruses are involved in a variety of diseases. Large-scale evaluation of the clinical and epidemiological importance of different herpesviruses requires high-throughput methods. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a method that has a multiplex capacity enabling simultaneous detection of several viruses in a single sample. PCR based methods for the multiplex detection of all known human herpesviruses were developed on the MALDI-TOF MS system. A variety of 882 archival samples, including bronchoalveolar lavage, conjunctival fluid, sore secretion, blister material, plasma, serum and urine, analysed for herpesviruses using PCR based reference methods, were used to evaluate the MALDI-TOF MS method. The overall concordance rate between the MALDI-TOF MS method and reference methods was 95.6% (κ 0.90). In summary, the MALDI-TOF MS method is well suited for large-scale detection of all known human herpesviruses in a wide variety of archival biological specimens.
Introduction

The herpesviruses infecting humans include herpes simplex virus (HSV) types 1 and 2, varicella-zoster virus (VZV), Epstein-Barr virus (EBV) types A and B, cytomegalovirus (CMV), human herpesvirus 6 (HHV6) types A and B, human herpesvirus 7 (HHV7) and human herpesvirus 8 (HHV8, Kaposi’s sarcoma associated herpesvirus, KSHV). Herpesvirus infections in healthy subjects are often asymptomatic but can cause oral and genital lesions (HSV), chicken pox or shingles (VZV), infectious mononucleosis (EBV and CMV (13)) or roseola (HHV6) (19).

In immunologically compromised hosts, herpes infections are more severe causing pneumonia (CMV (19) and HHV6 (1)), lymphoproliferative diseases (EBV) (19), Kaposi’s sarcoma (HHV8) (19) or encephalitis (HSV1) (6). Most herpesviruses have been reported to traverse the placenta (21) and intrauterine infections may cause birth defects (CMV) (19), premature delivery (VZV) (4) and foetal mortality (HSV) (21). Herpesvirus infections during pregnancy have also been associated with childhood acute lymphoblastic leukaemia (EBV) (14).

Common laboratory techniques in herpesvirus detection include antibody detection such as Enzyme-Linked ImmunoSorbent Assay (ELISA) (26), PCR (15, 17, 18, 25, 27) and dot blot hybridization (27). These techniques usually require separate methods for detection of each herpesvirus and clinical information for selection of virus assay. As most microbiological laboratories maintain large biobanks with clinical samples, large-scale evaluation of the clinical and epidemiological importance of different herpesviruses is today restricted only by cost and throughput considerations. There is therefore an increasing interest in rapid and large-scale herpesvirus detection and methods for the multiplex detection of several herpesviruses have recently been developed using PCR and microarray techniques (8, 10, 23). Virus detection using the MALDI-TOF mass spectrometry (MALDI-TOF MS) system is a high throughput technology...
with multiplex capacity that has been used for genotyping and detection of RNA viruses (3, 7, 9, 11, 12, 16) and human papillomavirus (HPV) (28).

The aims of this study were to develop an efficient screening method for qualitative multiplex detection of all human herpesviruses by MALDI-TOF MS and investigate the usefulness of a wide variety of archival sample types for human herpesvirus detection.

Material and Methods

Sample selection. A consecutive series of 860 archival aliquots of samples diagnostically tested for HSV1, HSV2, VZV, CMV or EBV (EBV Virus Capsid Antigen-IgM analysis) at the Department of Medical Microbiology, UMAS, Malmö was retrieved. All archived aliquots had been stored at -80°C or -20°C (EBV samples) for at least 18 months. For each positive sample, the negative sample, with the same type of biological material, submitted closest in calendar time was retrieved. Sample materials included bronchoalveolar lavage, conjunctival fluid, sore secretion, blister material, plasma, serum and urine (Table 1). Thirty-two of the selected samples had been analysed for 3 herpesviruses (HSV1, HSV2 and CMV (N=3) or HSV1, HSV2 and VZV (N=29)), 403 samples had been analysed for 2 herpesviruses (HSV1 and HSV2) and 425 samples had been analysed for 1 herpesvirus by clinical diagnostic testing. An additional 22 archived aliquots of serum samples that had tested positive for HHV8 specific antibodies, collected between 1996 and 2005, and stored at –80°C were obtained from the Department of Microbiology, Oncological Center, Aviano, Italy. Thus, a total of 1349 herpesvirus analyses had been performed on a total of 882 samples (Table 1).

The study was approved by the Institutional Review Board of Lund University.
Reference methods. Upon arrival at the Department of Medical Microbiology DNA extracted from an aliquot of each sample was analysed for the selected herpesvirus, the remainder of the sample was stored as described above.

(I) PCR analysis of HSV 1, HSV 2 and VZV. DNA was extracted from 200µL sample using a MagNA Pure LC instrument and Total Nucleic Acid Isolation Kit (Roche Diagnostics, Penzberg, Germany) and eluted in 100µL buffer, except for suspensions of VZV blister material for which DNA was released by heating to 95°C for 10 minute. The PCR methods were adapted from Aurelius et al (5) and Puchhammer-Stöckl et al (20). Briefly, 10µL MagNA extract, corresponding to 10 (VZV blister material) or 20µL original sample, was mixed with 1x Taq Buffer, 2mM MgCl$_2$, 0.2mM dNTP, 1U Taq polymerase and 0.4µM of each HSV outer primer (HSV1 (5), HSV2 Forward: 5’ TCA GCC CAT CCT CCT TCG GCA GTA 3’, Reverse: 5’ GAT CTG GTA CTC GAA TGT CTC CG 3’) or 28nM of each VZV primer (Forward: 5’ TCC ACG TAT GAC TCT CTC AC 3’, Reverse: 5’ GAT CAG ACA CAT GAC GAA TC 3’) in a 50µL reaction and a layer of mineral oil added. The PCR program included 93°C for 5 min, 20 cycles of 93°C for 50 s, 55°C for 50 s and 72°C for 60 s followed by 72°C for 5 min. Five µL of the primary PCR product was mixed with 1x Taq buffer, 2mM MgCl$_2$, 0.2mM dNTP, 0.4µM of each HSV inner primer (HSV1 (5), HSV2 Forward: 5’ AGA CGT GCG GGT CGT ACA CG 3’, Reverse: 5’ CGC GCG GTC CCA GAT CGG CA 3’) or 115nM of each VZV primer (20) and 1U Taq polymeras, in a 50µL reaction and a layer of mineral oil added. The PCR program was as above, extended by 10 cycles. The products were analysed by electrophoresis on a 2% agarose gel. All PCR reagents were from Applied Biosystems and primers form DNA-technology A/S (Aarhus C, Denmark).
(II) **CMV analysis.** DNA from 200µL sample was extracted and analysed using the PCR and hybridization based COBAS AMPLICOR CMV MONITOR™ Test and COBAS AMPLICOR™ Analyzer (Roche Diagnostics, Stockholm, Sweden). The template volume used in the PCR corresponds to 25µL original sample.

(III) **Qualitative EBV PCR** was performed on all samples serologically tested for EBV for clinical diagnosis, after DNA extraction of the archived aliquots as described below. Each 12µL reaction contained 4µL MagNa extract, corresponding to 16µL original sample, 5µL TaqMan PCR MasterMix (Applied Biosystems), 1mM additional MgCl₂ (Applied Biosystems), 900nM of each primer (Forward: 5’ AAG GTC AAA GAA CAA GGC CAA G 3’, Reverse: 5’ GCA TCG GAG TCG GTG GG 3’) (both CyberGene AB, Huddinge, Sweden). The PCR program included 95°C for 10 minutes followed by 50 cycles of 64°C for 1 minute and 95°C for 15 seconds. The PCR products were visualized on a 2% agarose gel as previously described (22) and compared to the size of a molecular weight marker (GeneRuler 50bp DNA ladder, MBI Fermentas) and EBV positive controls. The detection limit of the assay was 5 copies EBV control (prepared as below).

(IV) **Quantitative HHV8** analysis was performed on all samples serologically tested for HHV8, after DNA extraction as described below, by TaqMan real-time PCR as described by Tedeschi *et al* (24), using 2µL MagNA extract, corresponding to 4µL original sample, in a 20µl reaction supplemented with 0.1% BSA (A2153-50G, Sigma-Aldrich, Stockholm, Sweden) and 3.5mM MgCl₂ (Applied Biosystems) on a 7900 HT Sequence Detection System (Applied Biosystems). Samples with copy numbers below the lowest standard point detected were considered negative.

(V) **HHV6 and HHV7.** No reference methods were available for analysis of HHV6 and HHV7.

DNA extraction of archived sample aliquots. DNA from 200µL of each sample (100µL of HHV8 samples) was extracted using a MagNA Pure LC instrument and Total Nucleic Acid...
Isolation Kit (MagNA) and eluted in 50µL buffer. Three controls, 200µL each of NaCl, pooled HHV negative plasma (determined by the MALDI-TOF MS method) and pooled HHV negative plasma spiked with 25000 copies of Human Papillomavirus type 51 (HPV-51) control/mL were included in each batch of 32 samples. In the batch with HHV8 samples, 100µL of each control was used.

**Virus controls.** Viral particles were obtained from saliva from volunteers (HHV7) and the Swedish Institute for Infectious Disease Control in Solna, Sweden (HHV6A GS strain and HHV6B Z29 strain) and DNA was released by 2.5mg/mL proteinase K (AM2542, Applied Biosystems, Foster City, USA) incubation at 56°C overnight. Viral DNA and HPV-51 plasmid control were obtained from the department of Medical Microbiology, UMAS, Malmö (HSV1, HSV2, VZV, CMV and EBV A) and the Department of Microbiology, Oncological Center, Aviano, Italy (HHV8). Primary PCR amplicons, using appropriate primers (Table 2), were cloned into TOPO TA cloning vectors (Invitrogen, Carlsbad, USA). Specific sequences were confirmed to correspond to those reported in the NCBI database by DNA sequencing of the plasmids. No positive control was available for EBV B.

**MALDI-TOF MS analyses.** All procedures were performed according to SEQUENOM standard protocols unless otherwise specified. Two qualitative, PCR based, multiplex assays were designed to detect the human herpesviruses by MALDI-TOF MS (SEQUENOM MassARRAY, SanDiego, USA); one assay detecting HSV1, HSV2, CMV, EBV A, EBV B, VZV, and HHV8, the other detecting HHV6A, HHV6B and HHV7. The latter assay does not discriminate between HHV6A and HHV6B for the HST strain. Primer sequences, 5’- primer positions, target genes, NCBI reference sequence numbers, mass of unextended primers and mass and extension base of extended primers are presented in Table 2. The 10 base 5’ extension ACGTTGGATC –
recommended by SEQUENOM to increase the robustness in multiplex reactions was added to each forward and reverse primer. After optimization of annealing temperatures, and enzyme, MgCl₂ and primer concentrations, the SEQUENOM hME protocol was performed with the following adjustments: 2µL of MagNA extract, corresponding to 4 (HHV8) or 8µL original sample, was used in a 6µL primary PCR reaction, containing 1x PCR buffer, 200µM dNTP, 0.15U TaqGold, 3.5mM MgCl₂ (Applied Biosystems) and 0.5µM of each primer (Metabion, Martinsried, Germany). The PCR program included 95°C for 10 min, 5 cycles of 64°C for 30 s, 72°C for 60 s and 95°C for 30 s, 40 cycles of 72°C for 60 s and 95°C for 30 s followed by 72°C extension for 10 min. The PCR product was treated with shrimp alkaline phosphatase (SEQUENOM), 2µL secondary PCR mix (optimized for annealing temperature and primer concentration), containing 0.229µL ATC Terminator mix, 0.04µL MassExtend enzyme (SEQUENOM) and 1µM of each massextend primer (Metabion), was added and a secondary PCR, was performed at 94°C for 2 min followed by 99 cycles of 94°C for 5 s, 42°C for 5 s and 72°C for 6 s. After desalting by addition of 6mg clean resin to each sample, 15nL product of each sample was dispensed onto a 384-spot SpectroCHIP using a MassARRAY Nanodispenser (all SEQUENOM). The MALDI-TOF MS analysis was performed in a Bruker Autoflex (SEQUENOM), each sample was exposed to 9 individual laser pulses, the laser power was set to 35%, the linear detection voltage was 1588V and the time delay for ion extraction was 300nseconds, the spectra were acquired using SpectroACQUIRE. The MassARRAY Typer (SEQUENOM), used for interpretation of the results, is programmed primarily for single nucleotide polymorphism (SNP) genotyping. In contrast to SNP genotyping, in which abnormal peak height ratios generate “moderate” or “aggressive” calls, the clear identification of multiple peaks of appropriate mass, regardless of their height ratios, is interpreted as presence of all
corresponding viruses. Thus “conservative”, “moderate” and “aggressive” calls were interpreted as positive. “Low probability” and “no alleles” were judged negative.

**TaqMan real-time PCR.** All real-time PCR analyses were performed on a 7900 HT Sequence Detection System. HPV-51 (Forward primer: 5' GCG CAC TAA TGA CAG CAA GGT 3’, Reverse primer: 5’ CGG TGC GTG TGA TAT ATT CTT CTG 3’, Probe: 5'-FAM-TGC ACC TGT GTC TCG A-MGB-3’) and CMV (Primers and probe as described by Watzinger *et al* (25)) analyses were performed in 6µL reactions using TaqMan “assay by design” reagents (Applied Biosystems) and 2µL MagNA extract, supplemented with 0.1% BSA and 3.5mM MgCl₂. All MagNA extracts were from the same DNA extraction batch that was used in the MALDI-TOF MS analyses.

**Statistical methods.** Kappa (κ) (2) was calculated for the concordance between reference and MALDI-TOF MS methods (Table 3).

**Results**

**DNA extraction controls.** All controls, NaCl, HHV negative plasma and HHV negative plasma spiked with HPV-51, were analysed by HPV-51 real-time PCR. Mean viral yield (SD, N) of the plasma samples spiked with HPV-51 was 24.3% (16.8, 32). All other controls were negative for HPV-51.

**Detection limit of the MALDI-TOF MS method.** To determine the detection limit of the MALDI-TOF MS method, a 2µL aliquot from a dilution series of each HHV control was used as template in the MALDI-TOF MS method. Five copies of EBV, HHV6A and HHV6B control DNA were always detected (100% sensitivity). VZV and HHV7 had 100% sensitivity at 10 copies and HSV1, HSV2, CMV and HHV8 had 100% sensitivity at 100 copies. To determine the
detection limit for multiple infections, all 9 HHV controls were combined in a single mixture. The sensitivity for detecting all controls in a combined mixture containing 100 copies of each control was 78%. A typical example of the mass spectra resulting from a sample, in which 100 copies each of all controls were combined and successfully detected, is presented in Fig. 1. The specificity of the MALDI-TOF MS method, calculated using 100 copies of each HHV control as template, was 100% for HSV2, VZV, HHV8, HHV6B and HHV7, 98% for HSV1 and CMV, 95% for HHV6A and 92% for EBV. Analyses of dilution series were repeated a minimum of 7 times.

**The detection** limit of the entire analysis, including the DNA extraction step, was evaluated by the addition of serially diluted viral controls to HHV negative plasma prior to DNA extraction. At 2500 copies/mL plasma all HHV controls could be detected. Assuming 100% yield in the extraction process, 2500 copies/mL corresponds to 500 copies in the initial serum sample and 20 copies in the primary PCR template.

MALDI-TOF MS analyses. A variety of 882 archival samples that had been analysed for herpesviruses by reference PCR methods, 1349 analyses in total, were used to evaluate the MALDI-TOF MS method. The concordance rate between MALDI-TOF MS analyses, using template volumes corresponding to 4 (HHV8) or 8µL original sample, and reference methods, using template volumes corresponding to 4 (HHV8), 10 (VZV blister material), 16 (EBV), 20 (HSV, VZV) or 25 (CMV) µL original sample, was 95.6% (κ 0.90). A comparison of reference methods and MALDI-TOF MS results for each herpesvirus is presented in Table 3. Some (N=338) analyses were repeated up to 4 times. The results for 53 (15.7%) of the analyses fluctuated between runs, 39 (73.6%) of which were positive in the reference analysis. An analysis was considered positive if the same virus was identified in all or 3 of 4 analyses. Six samples that
were negative for HSV1 and HSV2 by the reference method were positive for VZV and 25 samples with negative VZV reference test were positive for HSV1 or HSV2 with the MALDI-TOF MS method. All of these samples consisted of blister material (N=29) or sore secretions (N=2). MALDI-TOF MS results in relation to the original clinical diagnostic request and multiple infections detected using the MALDI-TOF MS method are presented in Table 4. As the method does not discriminate between HHV6A and the HHV6B HST strain and all HHV6 positive samples were positive for HHV6A no discrimination between HHV6 types A and B is made in the table. All negative controls, including 2-24 non-template controls per analysis, were negative for all herpesviruses.

Real-time PCR. The viral load of CMV and HHV8 samples were analysed by real-time PCR, for respective viruses. All samples with negative or inconsistent CMV and HHV8 results using the MALDI-TOF MS method had viral loads below the detection limit of the MALDI-TOF MS method. Both samples that had been negative for CMV in reference tests but positive using the MALDI-TOF MS method were also positive for CMV in the real-time PCR.

Discussion

We have developed a multiplex MALDI-TOF MS method that successfully detects human herpesviruses in a wide variety of archival biological specimens. The concordance rate between the MALDI-TOF MS method, using template volumes corresponding to 4 or 8µL original sample, and reference methods, using template volumes corresponding to 4 – 25µL original sample, was 95.6% (κ 0.90). Consistently negative results for non-template controls and an overall 98% specificity for 100 copy plasmid controls validated this new method. By comparison,
a concordance rate of 94% was found in a previous report comparing PCR and oligonucleotide
microarray methods for multiplex herpesvirus detection (10).

Multiple infections, HHV6 and HHV7 were consistently demonstrated in repeated MALDI-TOF
MS analyses in a substantial proportion of the study samples. Although no reference method was
available for HHV6 and HHV7, DNA sequence validation of the plasmid controls, the unique
combination of primer sequences according to NCBI BLAST analysis and consistently positive
MALDI-TOF MS results for the plasmid controls demonstrate the validity of the method. As no
EBV B control virus was available, the accuracy of discrimination between EBV A and EBV B
must await further tests.

The detection limits of the MALDI-TOF MS method on all HHV controls were comparable to
previous reports of multiplex herpesvirus detection using an oligonucleotide microarray and
multiplex PCR techniques (8, 10). As all negative controls were negative and detection limits
could be documented with positive controls we judge fluctuations in results of some samples to
reflect low viral load near the detection limit of the method, rather than contamination, as shown
by real-time PCR quantitation for CMV and HHV8 samples. Low viral loads, or weak cross
reactivity in either the reference methods or the MALDI-TOF MS method, may also contribute to
the few discrepant analyses. Routine analysis of duplicate samples and clear indications of
sensitivity thresholds can be recommended for clinical use. Thus we believe that the multiplex
approach described here could be very useful for large-scale epidemiological research studies in
which broad multiplex human herpesvirus detection is of interest.

In some cases requested diagnostic testing for VZV was negative but the MALDI-TOF MS
analysis detected HSV – and vice versa. In other cases unsuspected viruses were demonstrated
alone or in combination with those that had been requested. This observation indicates the
difficulty in selecting the correct test based on clinical symptoms and suggests that a broad
multiplex human herpesvirus analysis could improve both the economy and the diagnostic accuracy of clinical viral testing. MALDI-TOF MS analyses have been reported for the detection of hepatitis- and human papillomaviruses (7, 9, 11, 12, 28). Our hospital, serving the southern region of Sweden, receives a sufficient number of samples per day to motivate 96 multiplex analyses for each of these three viral groups. Similar multiplex panels are being developed for human genetic disorders. Thus several diagnostic questions could be addressed on separate 96-well systems using 96- or 384 format spectroCHIPs. All MALDI-TOF MS reactions are performed in the same reaction plate, with robotic pipetting, with the added quality advantages of traceability of samples and reduced human error throughout the entire process.

In summary, the MALDI-TOF MS methods for multiplex human herpesvirus detection will allow large-scale research studies on archival samples of various biological materials. The MALDI-TOF MS methods may also become highly useful for multiplex clinical diagnostic testing, following validation by parallel analyses of patient samples with established standard methods.

Acknowledgement

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References


clinical samples comparison of PCR with standard laboratory methods for the


Table 1. Samples used in the study and results of reference methods.

<table>
<thead>
<tr>
<th>Clinical analysis</th>
<th>BAL&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Blister material</th>
<th>Conj. fluid&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Sore secretion</th>
<th>Plasma</th>
<th>Serum</th>
<th>Urine</th>
<th>Total no. analyses</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV1</td>
<td>28 (14)</td>
<td>370 (80)</td>
<td>18 (8)</td>
<td>19 (6)</td>
<td>435 (108)</td>
<td></td>
<td></td>
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<tr>
<td>HSV2</td>
<td>28 (0)</td>
<td>370 (106)</td>
<td>18 (1)</td>
<td>19 (3)</td>
<td>435 (110)</td>
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<tr>
<td>CMV</td>
<td>10 (5)</td>
<td></td>
<td></td>
<td>97 (48)</td>
<td>2 (1)</td>
<td>20 (10)</td>
<td>129 (64)</td>
<td></td>
</tr>
<tr>
<td>VZV</td>
<td></td>
<td>156 (78)</td>
<td>2 (1)</td>
<td>6 (3)</td>
<td></td>
<td></td>
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<td>164 (82)</td>
</tr>
<tr>
<td>EBV</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>164 (70)</td>
<td></td>
<td></td>
<td>164 (70)</td>
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<td>HHV8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>22 (12)</td>
<td></td>
<td></td>
<td>22 (12)</td>
</tr>
<tr>
<td>Total no. analyses</td>
<td>66 (19)</td>
<td>896 (264)</td>
<td>38 (10)</td>
<td>44 (12)</td>
<td>97 (48)</td>
<td>188 (83)</td>
<td>20 (10)</td>
<td>1349 (446)</td>
</tr>
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</table>

<sup>a</sup> Bronchoalveolar lavage, <sup>b</sup> Conjunctival fluid

The table shows total number of analyses, with number of positive analyses in parenthesis.
Table 2. Primers used in the MALDI-TOF MS analyses. All primer sequence directions are 5’ to 3’.

<table>
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<th>Primer name</th>
<th>Target gene</th>
<th>Primer sequence(^a)</th>
<th>NCBI ref seq</th>
<th>Primer 5’-position</th>
<th>UEP (Da)</th>
<th>Extension base(s)</th>
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<td></td>
<td>R: AACCTCATGAAGGTGCTGGAGTAC</td>
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<td>14075</td>
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<td><strong>CMV(^b)</strong></td>
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<td>716</td>
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<td><strong>EBV B</strong></td>
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<td>GGT</td>
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\(^a\) All primer sequences are 5’ to 3’.

\(^b\) CMV and VZV data from the literature.
<table>
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<tr>
<th>NCBI ref seq</th>
<th>NCBI database reference sequence number, (^a)</th>
<th>the 5’ extension ACGTTGGATG was added to all F and R primer, UEP unextended primer, EP extended primer, F forward, R reverse, ME massextend, (^b) F and R primers adapted from Mengoli et al (18)</th>
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<td>HHV6B</td>
<td>F: ATGAACCACGTTTTTCCATGTCTCT</td>
<td>103132</td>
</tr>
<tr>
<td>AF157706</td>
<td>R: GAACCATTTCAGAAGAACATTCTCATA</td>
<td>103331</td>
</tr>
<tr>
<td>HHV7</td>
<td>F: ATGAACCATGTGTTTACCGTGCTCTC</td>
<td>99572</td>
</tr>
<tr>
<td>AF037218</td>
<td>R: GAGCCATTTCAAAAGAGCATTCTTATAC</td>
<td>99771</td>
</tr>
<tr>
<td>U60</td>
<td>ME: AGCTTTCTGTGTTAAGTATG</td>
<td>99616 5824 T 6128</td>
</tr>
</tbody>
</table>
Table 3. Comparison of the MALDI-TOF MS (MS) results with reference PCR based methods (ref.).

<table>
<thead>
<tr>
<th>Analysis</th>
<th>No. samples</th>
<th>- ref. / - MS</th>
<th>+ ref. / + MS</th>
<th>- ref. / + MS</th>
<th>+ ref. / - MS</th>
<th>Concordance (%)</th>
<th>( \kappa )</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV1</td>
<td>435</td>
<td>325</td>
<td>98</td>
<td>2</td>
<td>10</td>
<td>97.2</td>
<td>0.92</td>
</tr>
<tr>
<td>HSV2</td>
<td>435</td>
<td>322</td>
<td>104</td>
<td>3</td>
<td>6</td>
<td>97.9</td>
<td>0.94</td>
</tr>
<tr>
<td>CMV</td>
<td>129</td>
<td>63</td>
<td>54</td>
<td>2</td>
<td>10</td>
<td>90.7</td>
<td>0.81</td>
</tr>
<tr>
<td>VZV</td>
<td>164</td>
<td>79</td>
<td>74</td>
<td>3</td>
<td>8</td>
<td>93.3</td>
<td>0.87</td>
</tr>
<tr>
<td>EBV</td>
<td>164</td>
<td>94</td>
<td>58</td>
<td>0</td>
<td>12</td>
<td>92.7</td>
<td>0.85</td>
</tr>
<tr>
<td>HHV8</td>
<td>22</td>
<td>10</td>
<td>9</td>
<td>0</td>
<td>3</td>
<td>86.4</td>
<td>0.73</td>
</tr>
<tr>
<td>All</td>
<td>1349</td>
<td>893</td>
<td>397</td>
<td>10</td>
<td>49</td>
<td>95.6</td>
<td>0.90</td>
</tr>
</tbody>
</table>
Table 4. MALDI-TOF MS results in relation to the original clinical diagnostic request.

<table>
<thead>
<tr>
<th>Analysis requested (no. samples)</th>
<th>HSV</th>
<th>HSV2</th>
<th>CMV</th>
<th>VZV</th>
<th>EBVA</th>
<th>EBVB</th>
<th>HHV8</th>
<th>HHV6</th>
<th>HHV7</th>
<th>2 HHV&lt;sup&gt;a&lt;/sup&gt;</th>
<th>3 HHV&lt;sup&gt;a&lt;/sup&gt;</th>
<th>4 HHV&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>HSV (435)</td>
<td>100</td>
<td>107</td>
<td>0</td>
<td>20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>11</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>21</td>
<td>16&lt;sup&gt;′&lt;/sup&gt;</td>
<td>1&lt;sup&gt;″&lt;/sup&gt;</td>
<td>1&lt;sup&gt;″&lt;/sup&gt;</td>
</tr>
<tr>
<td>CMV (129)</td>
<td>0</td>
<td>0</td>
<td>56</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>6&lt;sup&gt;′&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VZV (164)</td>
<td>14</td>
<td>16&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0</td>
<td>77</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>5&lt;sup&gt;h&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EBV (164)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>56</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>1&lt;sup&gt;i&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HHV8&lt;sup&gt;j&lt;/sup&gt; (22)</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>2&lt;sup&gt;k&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> 2, 3 or 4 herpesviruses detected in the same samples, also included in the column for respective virus

<sup>b</sup> 14 samples also submitted for VZV analysis

<sup>c</sup> 3 EBVA & HHV7, 1 VZV & HHV6, 2 EBVA & HSV1, 1 EBVA & HSV2, 1 HSV1 & HSV2, 2 HHV6 & HSV1, 6 HHV7 & HSV1

<sup>d</sup> HSV1, HHV6 & HHV7

<sup>e</sup> EBVA, EBVB, HSV1 & HHV7

<sup>f</sup> 2 EBVA & CMV, 1 EBVB & CMV, 1 HHV6 & CMV, 1 HSV1 & CMV, 1 HHV7 & CMV

<sup>g</sup> 3 samples also submitted for HSV analysis

<sup>h</sup> 3 HSV1 & HHV6, 1 HHV6 & VZV, 1 HSV1 & VZV, 1 EBV & HHV7

<sup>i</sup> EBV & HHV6

<sup>j</sup> not clinically diagnosed

<sup>k</sup> 1 HHV8 & CMV, 1 HHV8 & EBVA
Figure 1. MALDI-TOF MS spectra from a single sample containing 100 copies each of all 9 human herpesvirus controls.

UEP unextended primer