JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1994, p. 2434–2440
0095-1137/94/$04.00+0

Vol. 32, No. 10

Identification of Human Herpesvirus 6 Variants A and B by Amplimer Hybridization with Variant-Specific Oligonucleotides and Amplification with Variant-Specific Primers

JEAN-THIERRY AUBIN,* LAURENT POIREL, CATHERINE ROBERT, JEAN-MARIE HURAUX, AND HENRI AGUT

Laboratoire de Virologie–CNRS EP 57, C.E.R.V.I., Hôpital de la Pitié-Salpêtrière, 75013 Paris, France

Received 1 April 1994/Returned for modification 24 May 1994/Accepted 28 July 1994

Two distinct PCR-based procedures were evaluated for the detection and identification of human herpesvirus 6 (HHV-6) variants A and B in uncultured human samples. Variant-specific oligonucleotide hybridization (VSOH) is based on the amplification of two distinct regions of the HHV-6 genome, followed by hybridization of amplimers with variant-specific oligonucleotide probes. Variant-specific primer PCR (VSPP) is based on the amplification of each variant by using variant-specific primers. The study of 10 well-characterized HHV-6 strains allowed us to demonstrate the high sensitivity and specificity of both methods. With variant mixtures, however, some limitations of VSOH were evidenced and VSPP was required to obtain unambiguous results. The combination of VSOH and VSPP was applied to the direct study of 300 peripheral blood mononuclear cell samples from French subjects. HHV-6 was detected in 15 samples: 11 corresponded to variant B, 3 corresponded to variant A, and 1 corresponded to a mixture of both variants.

Human herpesvirus 6 (HHV-6) was first isolated in cultures of peripheral blood mononuclear cells (PBMCs) from patients with lymphoproliferative disorders (15). This virus was classified in the *Betaherpesvirinae* subfamily on the basis of its genetic organization, which is similar to that of human cytomegalovirus (12). HHV-6 has been found to be the causative agent of exanthem subitum (18). Primary infection is also associated with acute febrile illness without rash in young children (13). In contrast, HHV-6 has not been clearly related to any adult disease. Although seroprevalence rates vary from report to report, most seroepidemiological studies indicate a high frequency of infection in the general human population. Two groups of HHV-6 isolates have been phenotypically and genetically distinguished (1, 4, 16, 17) and are designated variants A and B, following an agreement among most HHV-6 researchers. HHV-6 variants A and B have been suspected to differ in pathogenicity (5, 16). In addition, in vivo coinfection with both HHV-6 variants has been previously described (9). For these reasons, it is useful to combine HHV-6 detection and variant identification to gain a better understanding of HHV-6 pathogenicity and epidemiology (2). Variant identification by *Hin*dIII digestion of amplified DNA has been previously described (4). However, it would be preferable to achieve detection and identification of HHV-6 by only a PCR approach without any additional procedure. In the present study, we investigated two distinct PCR approaches by using either variant-specific probes for the hybridization of amplimers or variant-specific primers for PCR. We demonstrated the usefulness of both approaches for detection and identification of HHV-6 variants A and B, in either a single or a mixed infection, when analyzing human cell samples.

* Corresponding author. Mailing address: Laboratoire de Virologie, CERVI, Hôpital Pitié-Salpétrière, 83, bd de l'hôpital, 75651 Paris Cedex 13, France. Phone: (33 1) 42 17 74 09. Fax: (33 1) 42 17 74 11.

MATERIALS AND METHODS

Viruses and cells. The 10 HHV-6 strains used in this study have been previously described (3). Four are variant A strains (SIE, TAN, GS, and U1102), and the six others are variant B strains (HST, BOU, MAR, BLE, MBE, and BLA). Viruses were propagated in phytohemagglutinin-stimulated PBMCs from healthy subjects, except for strain GS, which was propagated in the HSB-2 cell line. PBMCs and HSB-2 cells were tested for the absence of a positive signal in an HHV-6 PCR prior to HHV-6 infection. PBMCs were grown in RPMI medium supplemented with 10% heat-inactivated fetal calf serum containing 20 IU of recombinant interleukin 2 (Boehringer) per ml, 2 μg of Polybrene per ml, and 20 μg of both amikacin and vancomycin per ml. For DNA extraction, infected cells and culture supernatants were collected when a cytopathic effect was starting to develop (the mean time was between days 4 and 6 postinfection). For virus stocks, the supernatant from infected cell cultures was harvested when most of the cells showed a cytopathic effect, clarified by centrifugation at 2,500 × *g* for 10 min, and stored frozen at −80°C. Virus stocks were titrated by the endpoint dilution method previously reported (3), and infectious titers were expressed as 50% tissue culture-infective doses ($TCID_{50}$) per milliliter.

Three hundred individuals were included in our 1-year study of clinical samples. These subjects were initially investigated for the presence of retrovirus infection and subsequently tested for HHV-6 infection. The population studied included 125 children below 3 years of age and 175 adults whose ages ranged from 18 to 60. PBMCs were Ficoll purified from heparinized blood and counted, and a fraction of these cells was kept frozen until lysis.

Preparation of DNA. Cells were lysed in TE (10 mM Tris hydrochloride [pH 7.5], 1 mM EDTA) buffer containing 0.5% sodium dodecyl sulfate (SDS) and 200 μg of proteinase K per ml overnight at 37°C. Nucleic acids were extracted by phenol-chloroform treatment and ethanol precipitated.

Virus stocks were centrifuged at 2,500 × *g* for 30 min to pellet cell debris, and the supernatant was subsequently ultra-

TABLE 1. Oligonucleotides used in PCR assays

Location in HHV-6 genome	Oligonucleotide[a]	Variant specificity	Use in VSOH[b]	Use in VSPP[b]	Sequence (5′→3′)[c]
LTP gene	O10	A + B	Primer 1	Primer 1	GAT CCG ACG CCT ACA AAC AC
	O15	A + B	Primer 2	Primer 2	CGG TGT CAC ACA GCA TGA ACT CTC
	O12	A	Probe	NA[d]	GGC TGA TTA GGA TT**A** AT**A** GGA GA
	O212	B	Probe	NA	GGC TGA TTA GGA TT**C** AT**G** GGA GA
	O16	A	NA	Primer 1	**A**CT CGG AAT **G**AG GTC **A**AC TTC **T**
	O17	B	NA	Primer 2	GCA AAA **C**CA AGA AT**T** GTC C**AG**
	O18	A + B	NA	Probe	TAC CGC ATC CTT GAC ATA TTA CGC
MCP[e] gene	O22	A + B	Primer 1	NA	GCG TGA ATC AAA CCT CGC TCG A
	O23	A + B	Primer 2	NA	GCC TTA CTC GGA ATC TAC TGC
	O24	A	Probe	NA	CAG ATG **T**TC CAG ACA GGC AGC **G**C
	O224	B	Probe	NA	CAG ATG **C**TC CAG ACA GGC AGC **A**C

[a] Oligonucleotides O10, O15, and O12 were previously described as primers A, C, and S, respectively (3).
[b] Primer numbers 1 and 2 refer to the strands of genomic DNA to which the primers anneal.
[c] Boldface, underlined bases are sites critical for variant specificity.
[d] NA, not applicable.
[e] MCP, major capsid protein.

centrifuged at 435,000 × *g* for 10 min in a TLA100.2 rotor (Beckman). Pelleted virions were then lysed in a nonionic detergent buffer compatible with PCR conditions (10 mM Tris hydrochloride [pH 8.6], 50 mM KCl, 2.5 mM $MgCl_2$, 0.45% Tween 20, 0.45% Nonidet P-40, 200 μg of proteinase K per ml) and incubated for 2 h at 55°C. Proteinase K was subsequently heat inactivated at 95°C for 10 min. These lysates were directly submitted to PCR without nucleic acid extraction. For evaluation of PCR sensitivity, undiluted aliquots and serial dilutions of virus stocks were submitted to this DNA preparation procedure.

Detection and identification of HHV-6 variants by PCR and variant-specific oligonucleotide hybridization (VSOH). DNA samples were amplified for 40 cycles, each cycle consisting of denaturation at 92°C for 1 min, primer annealing at 55°C for 1 min, and chain elongation with 1.5 U of *Taq* polymerase (Beckman) at 72°C for 1 min. In the first cycle, samples were denatured at 94°C for 7 min, and in the last cycle, the extension step was increased to 7 min. The oligonucleotides used as PCR primers are described in Table 1. Oligonucleotides O10 and O15 were used to amplify an 830-bp DNA segment in the putative large tegument protein (LTP) gene, whereas oligonucleotides O22 and O23 were used to amplify a 380-bp DNA segment in the major capsid protein gene. The specificity of these two PCR assays has been previously demonstrated (3, 7). Amplification products were submitted to electrophoresis in a 1.5% agarose gel, stained with ethidium bromide, and blotted onto nylon membranes (Hybond N+; Amersham). The membranes were prehybridized for 1 h at 42°C in hybridization buffer containing 3× SSC (20× SSC is 3 M NaCl plus 0.3 M sodium citrate [pH 7]), 30% formamide, 0.5% SDS, 5× Denhardt's solution (0.1% Ficoll, 0.1% bovine serum albumin, 0.1% polyvinylpyrrolidone), and 100 μg of sonicated and heat-denatured salmon sperm DNA per ml. The oligonucleotides used as variant-specific probes are described in Table 1. For the O10-O15 amplimer (LTP gene), O12 and O212 were used as variant A-specific and variant B-specific probes, respectively. For the O22-O23 amplimer (major capsid protein gene), O24 and O224 were used as variant A-specific and variant B-specific probes, respectively. The sequences of these oligonucleotides were determined from cumulated sequencing data concerning the 10 HHV-6 isolates under study (3). Ten picomoles of each probe was 5′ end labelled with [γ-^{32}P]ATP (3,000 Ci/mmol) and 10 U of polynucleotide kinase (Boehringer). Hybridization was performed overnight at 42°C in the same buffer containing 10^6 cpm of each labelled probe per ml. The membranes were washed in a 2× SSC–0.5% SDS solution for 15 min at room temperature. Subsequently, membranes carrying O22-O23 amplimers were washed for 5 min at 60°C in a prewarmed 1× SSC–0.5% SDS solution, while those carrying O10-O15 amplimers were washed at 55°C for the same length of time. As a positive hybridization control, a set of membranes was probed with a mixture of the four probes under the same hybridization conditions, after which membranes were washed in 2× SSC–0.5% SDS for 15 min at room temperature and then in 1× SSC–0.5% SDS for 5 min at 50°C. Autoradiograms were analyzed after 48 h of exposure.

Detection and identification of HHV-6 variants by variant-specific primer PCR (VSPP). DNA samples were submitted to PCR with two primer pairs, O15-O16 and O10-O17, which were specific for variants A and B, respectively (Table 1). The two primer pairs were used to amplify two overlapping fragments of the LTP gene; the O15-O16 amplimer is 631 bp long, whereas the O10-O17 amplimer is 727 bp long. Annealing PCR steps were done at 63 and 60°C for O15-O16 amplification and O10-O17 amplification, respectively. A hot start procedure was used for O15-O16 amplification. A 2-μl volume of a 1× PCR buffer containing 1.5 U of *Taq* polymerase was added to each tube after 2 min of denaturation during the first cycle of amplification. All other parameters of the amplification reaction were identical to those of VSOH (see above). After electrophoresis and transfer, amplification products were hybridized as described above, by using the radiolabelled O18 oligonucleotide as a common detection probe. The HHV-6 genomic region complementary to the O18 sequence is identical for the 10 isolates studied. After the hybridization procedure, membranes were washed in 2× SSC–0.5% SDS for 15 min at room temperature and then in 1× SSC–0.5% SDS for 5 min at 50°C. As a positive control for HHV-6 detection, DNA samples were submitted to O10-O15 amplification and then O18 hybridization.

RESULTS

Specificity of VSOH and VSPP. DNA was extracted from PBMCs and HSB-2 cells infected with 10 reference HHV-6 strains and submitted to VSOH as described in Materials and Methods. The amount of DNA tested was 0.1 μg per reaction.

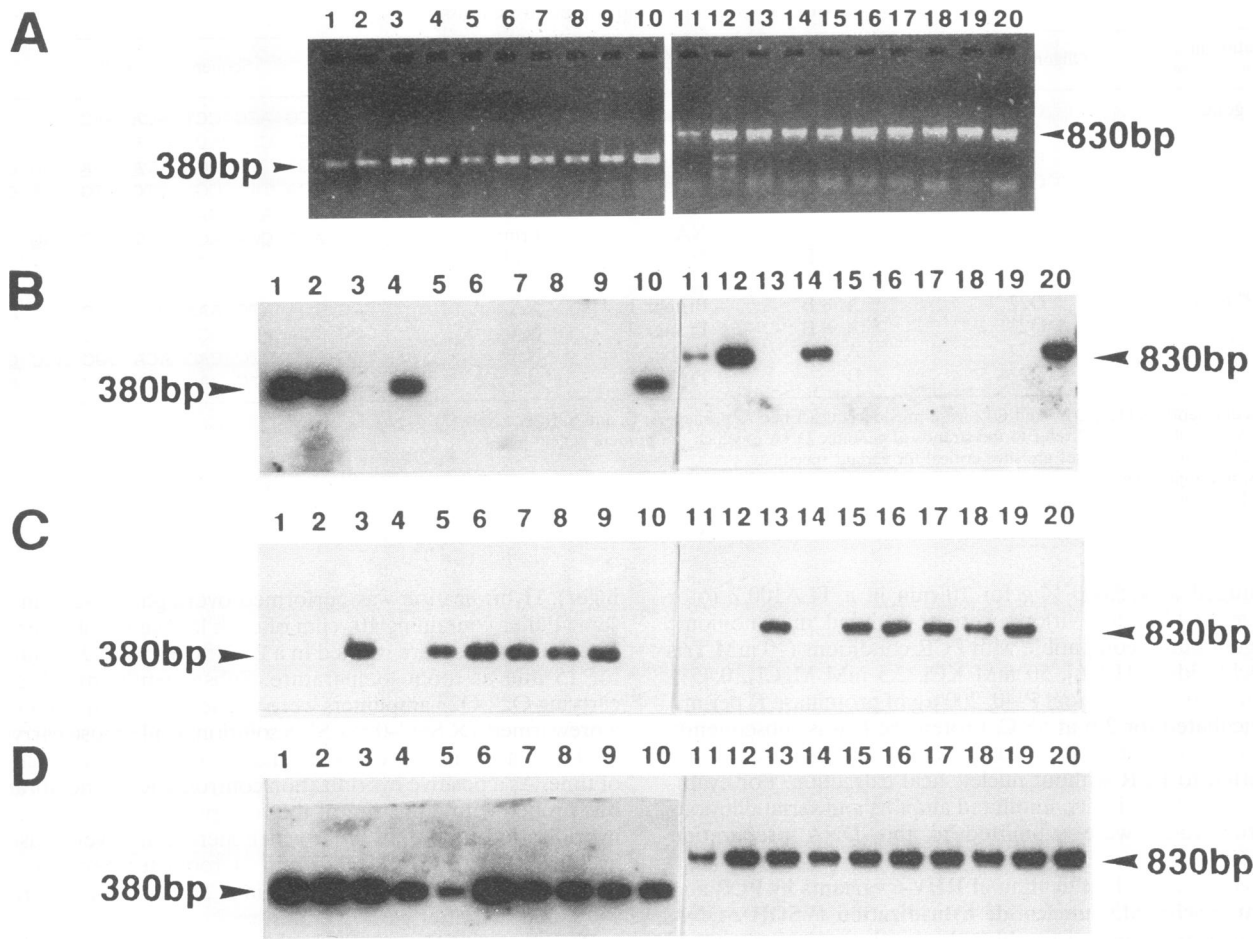

FIG. 1. VSOH applied to 10 HHV-6 strains. DNAs were extracted from HHV-6-infected cells and submitted to VSOH PCR as described in Materials and Methods. Lanes 1 to 10 correspond to the O22-O23 amplimers obtained from the following strains: 1, SIE; 2, TAN; 3, HST; 4, GS; 5, BOU; 6, MAR; 7, BLE; 8, MBE; 9, BLA; 10, U1102. Lanes 11 to 20 correspond to the O10-O15 amplimers obtained from the same strains in the same order. Panels: A, ethidium bromide staining of agarose gels; B, hybridization with probes O24 (lanes 1 to 10) and O12 (lanes 11 to 20); C, hybridization with probes O224 (lanes 1 to 10) and O212 (lanes 11 to 20); D, hybridization with a mixture of probes O24, O12, O224, and O212 (lanes 1 to 20).

Amplification was performed with both the major capsid protein and LTP genes by using the O22-O23 and O10-O15 primer pairs, respectively. As shown in Fig. 1, the corresponding 380- and 830-bp PCR products were detected by ethidium bromide staining for the 10 strains (panel A). Hybridization of O22-O23 amplimers with probe O24 (panel B, lanes 1 to 10) gave positive signals only with those obtained from strains SIE, TAN, GS, and U1102, belonging to the variant A group. Similar results were obtained when O10-O15 amplimers were hybridized with probe O12 (panel B, lanes 11 to 20). Conversely, hybridization of O22-O23 amplimers with the O224 probe and hybridization of O10-O15 amplimers with the O212 probe gave positive signals only with strains HST, BOU, MAR, BLE, MBE, and BLA, which belong to the variant B group (panel C). When the membranes were hybridized with a mixture of the four probes, clear positive signals were obtained for the 10 strains tested (panel D). These results were reproducibly obtained in other experiments with DNA from infected cells. The same results were also obtained with DNA corresponding to 10-μl volumes of clarified virus stocks (data not shown).

VSPP with the O15-O16 and O10-O17 primer pairs was applied to the 10 HHV-6 isolates by using the same amount of template DNA as for VSOH. In parallel, O10-O15 amplification was used as a positive control (Fig. 2). PCR with the O15-O16 primer pair gave 631-bp amplimers for isolates SIE, TAN, GS, and U1102 but failed to amplify the DNAs of strains HST, BOU, MAR, BLE, MBE, and BLA. This result, obtained after ethidium bromide staining (panel A), was confirmed after hybridization of amplimers with the O18 probe (panel D). Conversely, PCR with the O10-O17 primer pair gave a 727-bp amplimer for strains HST, BOU, MAR, BLE, MBE, and BLA but failed to amplify the DNAs of strains SIE, TAN, GS, and U1102 (panels B and E). With the O10-O15 primer pair in a parallel PCR, 830-bp specific amplimers were obtained for all of the strains (panels C and F). The same results were consistently obtained with DNA from clarified virus stocks (data not shown).

Sensitivity of VSOH and VSPP. As shown above, experiments with DNA extracted from either in vitro-infected cells or cell-free virus stocks provided large amounts of amplified products and unambiguous results with both the VSOH and VSPP methods. However, clinical samples might contain very small amounts of HHV-6 DNA and consequently lead to small

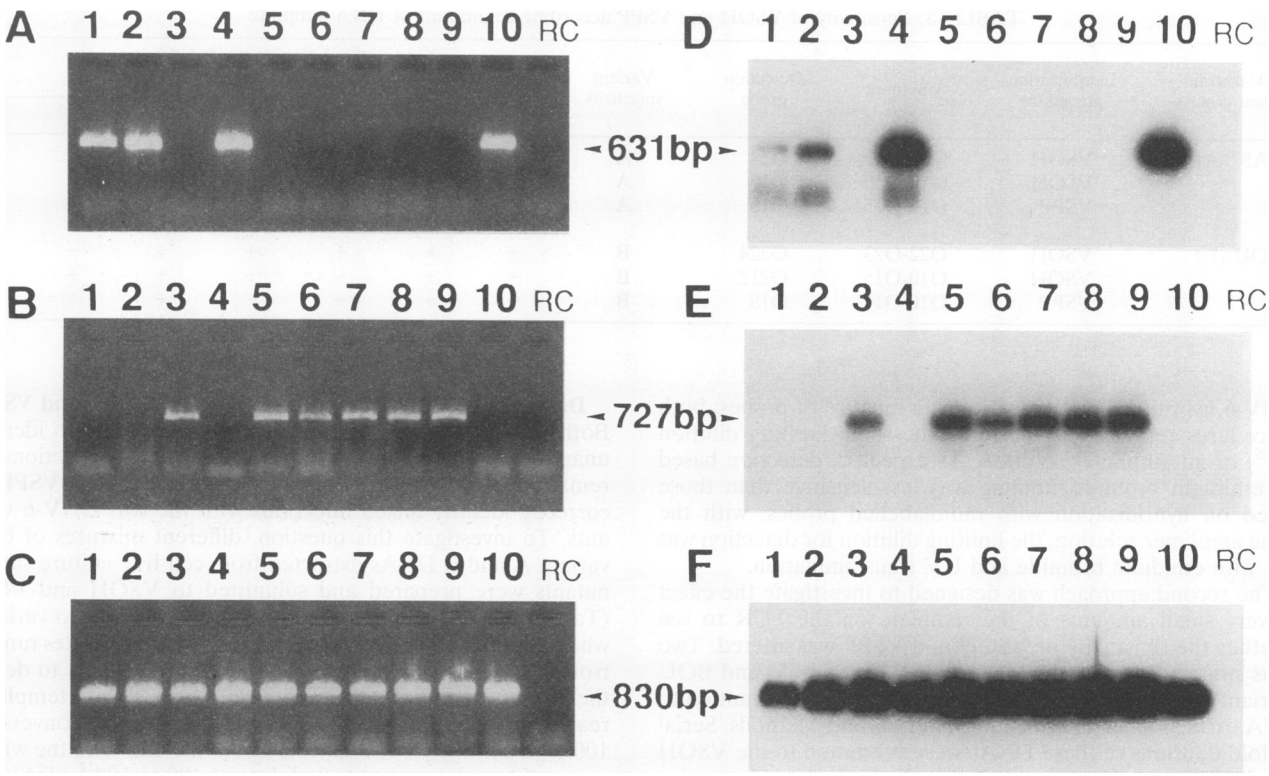

FIG. 2. VSPP applied to 10 HHV-6 strains. DNAs were extracted from HHV-6-infected cells and submitted to VSPP as described in Materials and Methods. The 10 HHV-6 strains tested were the same as in Fig. 1, in the same order from lanes 1 to 10. Panels: A, B, and C, ethidium bromide staining of agarose gels; D, E, and F, hybridization with probe O18; A and D, VSPP with O15-O16; B and E, VSPP with O10-O17; C and F, PCR with O10-O15. RC, reagent control.

amounts of amplified products. To prove that the sensitivity of both variant-specific procedures was as high as that of variant-unspecific HHV-6 PCR, we used two distinct approaches. In the first approach, serial fivefold dilutions of O22-O23 and O10-O15 amplimers obtained from either the TAN (variant A) or the BOU (variant B) strain were analyzed by VSOH. The membranes were hybridized first with a single variant-specific probe and subsequently with a mixture of variant-specific probes (similar to the control reaction described in Materials and Methods and used in previous experiments [Fig. 1D]). The results of this experiment (Table 2) indicated that variant-specific hybridization was as sensitive as the variant-unspecific

TABLE 2. Sensitivity of VSOH according to the amount of amplified products

HHV-6 strain (variant group)	Amplimer (size [bp])	Detection procedure[a]	Probe(s)	Hybridization specificity	Result of detection at following amplimer dilution:						
					Undiluted	5^{-1}	5^{-2}	5^{-3}	5^{-4}	5^{-5}	5^{-6}
TAN (A)	O22-O23 (380)	EtBr	NA[b]	NA	+	+	+	−	−	−	−
		Hybr.	O24	A	+	+	+	+	+	+	−
		Hybr.	O24-O224	A + B	+	+	+	+	+	+	−
	O10-O15 (830)	EtBr	NA	NA	+	+	+	−	−	−	−
		Hybr.	O12	A	+	+	+	+	+	+	−
		Hybr.	O12-O212	A + B	+	+	+	+	+	+	−
BOU (B)	O22-O23 (380)	EtBr	NA	NA	+	+	+	−	−	−	−
		Hybr.	O224	B	+	+	+	+	+	+	−
		Hybr.	O24-O224	A + B	+	+	+	+	+	+	−
	O10-O15 (830)	EtBr	NA	NA	+	+	+	−	−	−	−
		Hybr.	O212	B	+	+	+	+	+	+	−
		Hybr.	O12-O212	A + B	+	+	+	+	+	+	−

[a] EtBr, amplimer detection after agarose gel electrophoresis, staining with ethidium bromide, and UV transillumination; Hybr., amplimer detection after hybridization with the indicated ^{32}P-labelled probe(s) and autoradiography.
[b] NA, not applicable.

TABLE 3. Sensitivity of VSOH and VSPP according to amount of DNA template

HHV-6 strain (variant group)	Identification procedure	Amplimer	Detection probe	Variant specificity	Amplimer detection with following amt ($TCID_{50}$ equivalents) of DNA template:						
					1	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}
TAN (A)	VSOH	O22-O23	O24	A	+	+	+	+	+	−	−
	VSOH	O10-O15	O12	A	+	+	+	+	+	+	−
	VSPP	O16-O15	O18	A	+	+	+	+	+	+	−
BOU (B)	VSOH	O22-O23	O224	B	+	+	+	+	+	−	−
	VSOH	O10-O15	O212	B	+	+	+	+	+	+	−
	VSPP	O10-O17	O18	B	+	+	+	+	+	+	−

HHV-6 hybridization obtained with a mixture of probes: both procedures reproducibly detected the same limiting dilution (5^{-5}) of an amplimer solution. As expected, detection based on ethidium bromide staining was less sensitive than those based on hybridization with radiolabelled probes: with the same amplimer solution, the limiting dilution for detection was 5^{-2} with ethidium bromide and UV translumination.

The second approach was designed to investigate the effect of very small amounts of the template on the PCR to see whether the sensitivity of VSOH and VSPP was altered. Two virus stocks, corresponding to the TAN (variant A) and BOU (variant B) strains, respectively, were titered and submitted to DNA extraction as described in Materials and Methods. Serial 10-fold dilutions of these DNAs were submitted to the VSOH and VSPP procedures to determine the endpoint dilution for homologous detection. Both VSOH and VSPP were found to be highly and equally sensitive for variant identification (Table 3): DNA amounts equivalent to 10^{-5} $TCID_{50}$ were detected and correctly identified by either VSOH with O10-O15 amplimers or VSPP, for variant A as well as for variant B. However, with VSOH with O22-O23 amplimers, the smallest DNA amount detected and identified was reproducibly equivalent to 10^{-4} $TCID_{50}$ for both variants. The decreased sensitivity of VSOH with O22-O23 amplimers compared with either VSOH with O10-O15 amplimers or VSPP was due to a lower efficacy of PCR with primers O22 and O23 when the amount of the DNA template was a limiting factor. Despite this limitation, VSOH with primers O22 and O23 remained about 10,000-fold more sensitive than culture for detection of HHV-6.

In parallel, DNA extracted from serial 10-fold dilutions of virus stocks provided similar endpoint results (not shown), confirming both the high sensitivity of our assays and the convergence between the data obtained with DNA dilution and those obtained with virus stock dilution.

Detection of HHV-6 variant mixtures by VSOH and VSPP. Both the VSOH and VSPP procedures allowed us to identify unambiguously either variant A or B in single infections. A remaining question was the ability of VSOH and VSPP to correctly identify mixed infections with the two HHV-6 variants. To investigate this question, different mixtures of both variant A and B DNAs extracted from cell-free culture supernatants were prepared and submitted to VSOH and VSPP (Table 4). VSOH recognized the presence of the two variants when the ratio of variant A to variant B DNA templates ranged from 1:5 to 5:1. However, this technique was unable to detect the presence of both variants when the ratio of templates reached more extreme values, such as 1:100 or, conversely, 100:1. In contrast, VSPP detected both variants with the whole range of template ratios tested, from 1:100 to 100:1 (Table 4).

Application to clinical samples. The VSOH and VSPP procedures were applied to 300 different PBMC samples, 15 of which had been previously found positive with conventional HHV-6 PCR (4). Samples were encoded with novel designations and blindly tested. The amounts of DNA tested ranged from 0.1 to 1 μg per reaction. Both VSOH and VSPP detected the presence of HHV-6 DNA in the 15 samples which had been previously found to be positive. Regarding variant identification, 11 samples corresponded to variant B infections and 3 corresponded to variant A infections. Concordant results were obtained by both procedures for these 14 samples. In addition, virus isolation had been obtained in parallel from two of these samples by using PBMC coculture. The two isolates were characterized as two variant B isolates on the basis of both reactivity to monoclonal antibodies and the restriction pattern of amplified products (3), in agreement with the VSOH and VSPP findings.

Of the 15 HHV-6-positive samples, 1 corresponded to a variant B infection according to VSOH results and to a mixed variant A-variant B infection according to VSPP results. The

TABLE 4. Application of VSOH and VSPP to detection of a mixture of HHV-6 variants

Detection procedure	Amplimer	Probe(s)	Variant specificity	Result of detection in variant mixtures with following ratio of TAN-BOU PCR units[a] in reaction:						
				1:0	1:100	20:80	50:50	80:20	100:1	0:1
VSOH	O10-O15	O12	A	+	−	+	+	+	+	−
	O10-O15	O212	B	−	+	+	+	+	−	+
	O10-O15	O12-O212	A + B	+	+	+	+	+	+	+
VSPP	O16-O15	O18	A	+	+	+	+	+	+	−
	O17-O10	O18	B	−	+	+	+	+	+	+
	O10-O15	O18	A + B	+	+	+	+	+	+	+

[a] One PCR unit was defined as the smallest amount of DNA reproducibly giving detectable O10-O15 amplimers after ethidium bromide staining of an electrophoresis gel.

*Hin*dIII restriction pattern of O10-O15 amplimers did not provide convincing proof of the presence of both variants. A second PBMC sample was obtained from the same subject 3 months later and submitted to VSOH and VSPP analyses. The same results were obtained, indicating that a mixed HHV-6 infection with a high ratio (probably higher than 5:1) of variant B DNA to variant A DNA was present in both samples.

DISCUSSION

Most epidemiological studies on the distribution of HHV-6 variants are based on genotypic characterization of either virus isolates or PCR-amplified DNA. HHV-6 isolation by means of PBMC coculture is time consuming and not often possible, even when the procedure is specifically targeted for HHV-6 detection. Moreover, PBMC culture could favor the selection of one variant in mixed infections. PCR is an alternative method which can be substituted for cell culture, but it presents other pitfalls. The risk of false-positive results due to amplified product carryover is of special importance, since detection of this contamination is difficult in the absence of viral isolation. The risk of false-negative results must be also considered. When the amount of amplimers is small, hybridization with HHV-6-specific radiolabelled oligonucleotides generally permits their detection but may fail in some cases if the probe and amplimer sequences are too divergent. This can occur if the probe sequence has been fortuitously selected in highly polymorphic regions of the viral genome. The recent description of variant mixtures raises additional questions about the PCR approach (9). The identification of both variants from HHV-6 amplified products by either restriction analysis or variant-specific oligomer hybridization implies that amplimers from each variant are fairly equally represented among total amplified products. This may not be true following PCR amplification, which tends to increase the imbalance between variants that already exist in the initial sample, in the sense that minority genotypes representing roughly less than 5% of the total virus population could not be detected after PCR, as reported by Dewhurst and coworkers (9) and confirmed by our results. Moreover, either partial digestion of amplimers or high-level background oligonucleotide hybridization signals might be misinterpreted as variant mixture detection.

We have investigated two different approaches to variant identification by PCR, i.e., VSOH and VSPP. Both are highly sensitive and specific. The opportunity to use two separate PCR amplifications targetting two different genes in the VSOH procedure is interesting because it minimize the occurrence of false-positive results due to PCR carryover and because variant identification is based on converging results from both reactions. In our study, the use of variant-specific probes shorter than 20 bases and containing the same number of critical sites for variant specificity permitted even more ready discrimination between variants but greatly reduced the sensitivity of the hybridization process (data not shown). In mixtures of variants A and B, VSOH was not able to detect a minor variant; VSPP provided much better results. Since *Taq* polymerase lacks 3′-exonuclease activity, the specificity of DNA strand elongation with primers with critical modifications at the 3′ end permits detection of the corresponding variant strain even in the presence of an excess amount of the other variant. Given this high specificity, VSPP might also be applied to the quantitation of each HHV-6 variant in the same sample; experiments intended to demonstrate this possibility are in progress. The usefulness of VSPP as a first-line strategy for HHV-6 diagnosis remains to be proven. VSPP requires two distinct PCR assays done under slightly different conditions, while VSOH is based on a single initial PCR. Screening of a large number of specimens with a low frequency of HHV-6 positivity would be easier with VSOH. Conversely, accurate characterization of HHV-6-positive specimens must include VSPP. VSOH and VSPP must be considered complementary approaches to HHV-6 diagnosis and variant identification. Our data confirmed those of previous studies of HHV-6 variant identification, which were based on similar approaches. Drobyski and coworkers used sequence-specific oligonucleotide probe hybridization of amplimers in the presence of tetramethylammonium chloride (10), while Chou and Marousek based their discrimination between the two variants upon the difference in length between amplified products (6). However, those investigators did not address the ability of these two procedures to detect both variants in mixtures with a great imbalance between the variants, a crucial point in view of our results.

The frequency of HHV-6 detection in DNA extracted from uncultured PBMCs was 5% in our study. This frequency can be considered low compared with the frequency of 90% reported by Cone and coworkers (8). We had previously shown that the sensitivity of PCR with primers O10-O15 and radiolabelled probe O12 detected 50 copies of the target sequence as determined with serial dilutions of plasmid pHC5 (7). With infected-cell DNA, we obtained a positive result with 5 fg of total DNA, which is a much amount of DNA than that in a single cell (7). In the present study, the limit of detection was determined by using serial dilutions of titered virus stocks in order to have the same approach for any HHV-6 isolate and to test genomic HHV-6 DNA in a more relevant context than recombinant plasmids. A DNA amount equivalent to 10^{-4} to 10^{-5} $TCID_{50}$ could be detected, a sensitivity threshold close to that reported by others (8, 11). By using a very sensitive nested PCR assay, Rajcani and coworkers recently detected HHV-6 DNA in only 3.5% of leukocytes from healthy Slovak subjects (14). The wide range of HHV-6 frequencies detected in PBMCs (3.5 to 90%) suggests differences both in the amount of DNA tested and in the rate of active HHV-6 infection among the populations studied rather than striking differences in sensitivity between PCR assays.

Our results concerning the distribution of HHV-6 variants in specimens from a French population are in full agreement with the results of studies of European and American populations (5, 9). We detected variant B the most frequently in PBMCs (12 of 15 cases). However, recent data indicate that HHV-6 variant distribution may vary in the different body compartments (10a), suggesting a putative different cell tropism. In this context, exclusive analysis of PBMCs for determination of HHV-6 infection status must be questioned. The possibility of detecting more variant A infections than initially reported must be investigated by using more sophisticated approaches and a wider range of specimens. In addition, the detection of mixed variant A-variant B infections in vivo indicates that infections by either variant are not mutually exclusive in humans. These findings might lead to novel studies of HHV-6 epidemiology and pathogenicity. For that purpose, precise variant identification by procedures such as VSOH and VSPP would provide essential information.

ACKNOWLEDGMENTS

We thank K. Yamanishi for the gift of strain HST, S. Z. Salahuddin for the gift of strain GS, Hélène Collandre for constant help and interest in our work, Brigitt Griffith for critical review of the manuscript, and Anne-Marie Fillet and Richard Cone for stimulating discussions.

This study was partly supported by grants from the Agence Nationale de Recherche sur le SIDA, the Association Claude Bernard, the Ministry of Higher Education and Research, and the Groupe de Réflexion et d'Etude sur le Contrôle des Infections Virales.

REFERENCES

1. **Ablashi, D. V., N. Balachandran, S. F. Josephs, C. L. Hung, G. R. F. Krueger, B. Kramarsky, S. Z. Salahuddin, and R. C. Gallo.** 1991. Genetic polymorphism, growth properties, and immunologic variations in human herpesvirus-6 isolates. Virology **184:**545–552.
2. **Agut, H.** 1993. Puzzles concerning the pathogenicity of human herpesvirus 6. N. Engl. J. Med. **329:**203–204.
3. **Aubin, J. T., H. Agut, H. Collandre, K. Yamanishi, B. Chandran, L. Montagnier, and J. M. Huraux.** 1993. Antigenic and genetic differentiation of the two putative types of human herpes virus 6. J. Virol. Methods **41:**223–234.
4. **Aubin, J. T., H. Collandre, D. Candotti, D. Ingrand, C. Rouzioux, M. Burgard, S. Richard, J. M. Huraux, and H. Agut.** 1991. Several groups among human herpesvirus 6 strains can be distinguished by Southern blotting and polymerase chain reaction. J. Clin. Microbiol. **29:**367–372.
5. **Bovenzi, P., P. Mirandola, P. Secchiero, R. Strumia, E. Cassai, and D. Di Luca.** 1993. Human herpesvirus 6 (variant A) in Kaposi's sarcoma. Lancet **341:**1288–1289.
6. **Chou, S., and G. I. Marousek.** 1994. Analysis of interstrain variation in a putative immediate-early region of human herpesvirus 6 DNA and definition of variant-specific sequences. Virology **198:**370–376.
7. **Collandre, H., J. T. Aubin, H. Agut, J. M. Bechet, and L. Montagnier.** 1991. Detection of HHV-6 by the polymerase chain reaction. J. Virol. Methods **31:**171–180.
8. **Cone, R. W., M. L. W. Huang, R. Ashley, and L. Corey.** 1993. Human herpesvirus 6 DNA in peripheral blood cells and saliva from immunocompetent individuals. J. Clin. Microbiol. **31:**1262–1267.
9. **Dewhurst, S., B. Chandran, K. MacIntyre, K. Schnabel, and C. B. Hall.** 1992. Phenotypic and genetic polymorphisms among human herpesvirus 6 isolates from North America infants. Virology **190:**490–493.
10. **Drobyski, W. R., M. Eberle, D. Majewski, and L. A. Baxter-Lowe.** 1993. Prevalence of human herpesvirus 6 variant A and B infections in bone marrow transplant recipients as determined by polymerase chain reaction and sequence-specific oligonucleotide probe hybridization. J. Clin. Microbiol. **31:**1515–1520.

10a. **Fillet, A.-M., and R. Cone.** Personal communication.

11. **Kondo, K., Y. Hayakawa, H. Mori, S. Sato, T. Kondo, K. Takahashi, Y. Minamishima, M. Takahashi, and K. Yamanishi.** 1990. Detection by polymerase chain reaction amplification of human herpesvirus 6 DNA in peripheral blood of patient with exanthem subitum. J. Clin. Microbiol. **28:**970–974.
12. **Lawrence, G. L., M. Chee, M. A. Craxton, U. A. Gompels, R. W. Honess, and B. G. Barrell.** 1990. Human herpesvirus 6 is closely related to human cytomegalovirus. J. Virol. **64:**287–299.
13. **Pruksananonda, P., C. Breese Hall, R. A. Insel, K. McIntyre, P. E. Pellett, C. E. Long, K. C. Shnabel, P. H. Pincus, F. R. Stamey, T. R. Dambaugh, and J. A. Stewart.** 1992. Primary human herpesvirus 6 infection in young children. N. Engl. J. Med. **326:**1445–1450.
14. **Rajcani, J., R. Yanagihara, M. S. Godec, J. W. Nagle, M. Kudelova, and D. M. Asher.** 1994. Low-incidence latent infection with variant B or roseola type human herpesvirus 6 in leukocytes of healthy adults. Arch. Virol. **134:**357–368.
15. **Salahuddin, S. Z., D. V. Ablashi, P. D. Markham, S. F. Josephs, S. Sturzenegger, M. Kaplan, G. Halligan, P. Biberfeld, F. Wong-Staal, B. Kramarsky, and R. C. Gallo.** 1986. Isolation of a new virus, HBLV, in patients with lymphoproliferative disorders. Science **234:**596–600.
16. **Schirmer, E. C., L. S. Wyatt, K. Yamanishi, W. J. Rodriguez, and N. Frenkel.** 1991. Differentiation between two distinct classes of viruses now classified as human herpes virus 6. Proc. Natl. Acad. Sci. USA **88:**5922–5926.
17. **Wyatt, L. S., N. Balachandran, and N. Frenkel.** 1990. Variations in replication and antigenic properties of human herpesvirus 6 strains. J. Infect. Dis. **162:**852–857.
18. **Yamanishi, K., T. Okuno, K. Shiraki, M. Takahashi, T. Kondo, Y. Asano, and T. Kurata.** 1988. Identification of human herpes virus 6 as causal agent for exanthem subitum. Lancet **i:**1065–1067.