

## Analysis of Human Papillomavirus Types in Exophytic Condylomata Acuminata by Hybrid Capture and Southern Blot Techniques

DARRON R. BROWN,<sup>1,2\*</sup> JANINE T. BRYAN,<sup>3</sup> HARVEY CRAMER,<sup>4</sup> AND KENNETH H. FIFE<sup>1,3,4</sup>

*Division of Infectious Diseases, Department of Medicine,<sup>1</sup> Department of Pathology,<sup>4</sup> and Department of Microbiology and Immunology,<sup>3</sup> Indiana University School of Medicine, and Richard L. Roudebush Veterans Administration Medical Center,<sup>2</sup> Indianapolis, Indiana 46202*

Received 1 April 1993/Returned for modification 27 May 1993/Accepted 15 July 1993

**Exophytic condylomata acuminata of the external genitalia of 40 patients were analyzed for human papillomavirus (HPV) DNA by the Southern blot and hybrid capture methods. All lesions were initially analyzed by the Southern blot method by using a mixture of HPV type 6, 11, 16, and 18 whole genomic probes. Southern blots demonstrated characteristic *Pst*I restriction patterns of HPV type 6, 11, or 16 in all but one lesion. HPV 6 subtypes accounted for 28 of 39 HPV-positive lesions. Twenty-seven of these 28 lesions contained HPV type 6a, and 1 lesion contained HPV type 6c. Eight lesions contained HPV type 11 and three contained HPV type 16. Two of the three condylomata acuminata containing HPV type 16 were obtained from solid-organ transplant recipients receiving immunosuppressive medications. The third lesion containing HPV type 16 was a typical exophytic condyloma acuminatum from a woman with previously resected vulvar carcinoma. The hybrid capture assay detected HPV DNAs in all lesions except the Southern blot-negative lesion. Twenty-five lesions were positive for the A probe only (HPV types 6 and 11 and related types). All of these lesions were found to contain HPV type 6 or 11 sequences in the Southern blot assay. The remaining 14 lesions were positive for both the A probe and the B probe (HPV types 16 and 18 and related types). The strongest signal in these 14 lesions by the hybrid capture assay was consistent with the result of the Southern blot assay in all but one case. We conclude that (i) HPV type 6a is the most common type found in these lesions, (ii) HPV type 16 may be present more often in exophytic condylomata acuminata from immunosuppressed individuals, (iii) hybrid capture is a useful tool for documenting the presence of HPV sequences in DNAs from exophytic condylomata acuminata, and (iv) in samples containing multiple HPV types, hybrid capture allows detection of minority HPV types.**

Condylomata acuminata, or genital warts, are caused by infection of genital epithelial surfaces with human papillomaviruses (HPVs) (5). Of the 66 known HPV types, approximately 14 regularly infect the genital tract, causing a range of manifestations from asymptomatic, latent infection, to the typical exophytic cauliflower-like growths known as condylomata acuminata, to dysplasia and invasive carcinoma. The majority of condylomata acuminata, a benign condition, contain HPV type 6 (HPV-6) or HPV-11 genomes. HPV-6 has been detected approximately three times as often as HPV-11 in these lesions (10). Together, HPV types 6 and 11 have been detected in up to 86% of condylomata acuminata (10). These HPV types are thought to be of low malignant potential, although some studies have demonstrated the presence of HPV-6 or HPV-11 in malignant genital lesions, especially of the vulva (12, 22). HPV types 16 and 18 are more often associated with malignant genital lesions and have not been commonly detected in exophytic condylomata acuminata of the penis or vulva (18). In contrast to exophytic condylomata acuminata of external genitalia, condylomata acuminata of the uterine cervix contain HPV type 16 or 18 in approximately 20% of women (16). Infection of the cervix with HPV type 16 or 18 is associated with the rapid development of cervical dysplasia in a high percentage of women (13). It may be important to establish the presence of HPV type 16 or 18 in condylomata acuminata. A high percentage

of women with vulvar HPV infections also have cervical infections, possibly with the same HPV type (6, 23, 24), although the result of one study disagreed with this finding (9).

HPV-6 has been subtyped on the basis of the restriction digestion patterns visualized in Southern blots (10). There may be functional differences between the HPV-6 subtypes (25), which have been designated HPV-6a through HPV-6f. In addition, knowledge of the specific subtype may be useful in epidemiologic studies. HPV-6b is considered to be the "prototype" of HPV-6, perhaps because it was the first to be cloned and sequenced (11). HPV-6b DNA has been widely used in research, largely because of the generosity of the individuals who originally cloned the genome.

Gissmann et al. (10) detected HPV-6a in 26 of 37 condylomata acuminata found to contain HPV-6 sequences. It is not clear how often the HPV-6 subtypes are detected in condylomata acuminata from patients in the United States, as opposed to Germany, where the previous study was performed. It is also not clear whether patients who are pregnant or have conditions known to affect cell-mediated immunity have different HPV types in typical exophytic condylomata acuminata. To address these questions, we analyzed DNAs extracted from 40 exophytic condylomata acuminata of the penis, vulva, or perianal areas from 11 men and 29 women. Some of the patients were immunosuppressed, and eight of the women were pregnant at the time of biopsy. The Southern blot method was compared with the hybrid capture method for characterizing the HPV types and

\* Corresponding author.

subtypes present in the lesions. Hybrid capture is a recently developed assay that has not been extensively tested in detecting HPV in exophytic condylomata acuminata, but it offers a potential alternative method that may have several advantages over the Southern blot method.

## MATERIALS AND METHODS

**Excision biopsies.** Biopsies of typical exophytic condylomata acuminata were performed as described previously (4). Informed consent was obtained from all patients. Samples were held in normal saline until processing occurred, which was generally within 2 h. The biopsy samples of the exophytic lesions were 3 by 3 by 2 mm or larger. Lesions were split into two equal fragments. The first fragment, to be used for extraction of DNA, was frozen in liquid nitrogen. The second fragment was placed in 10% buffered formalin to prepare paraffin-embedded sections. One section from each sample was deparaffinized and stained with hematoxylin and eosin for confirmation of histology consistent with papillomavirus infection.

**Extraction of DNA.** Biopsy samples were frozen in liquid nitrogen and were then processed with a Braun mikrodismembrator II (B. Braun Instruments, Melsungen, Germany). The resulting material was solubilized in a buffer containing 10 mM EDTA and 0.6% sodium dodecyl sulfate (SDS). Proteinase K was added at 50 µg/ml for 90 min at 60°C. Following extraction with phenol and then chloroform-isoamyl alcohol, DNA was precipitated with sodium acetate and ethanol. The DNA concentration was determined by spectrophotometry. The presence of high-molecular-weight DNA was established by agarose gel electrophoresis and then staining with ethidium bromide.

**Hybrid capture assay.** The presence of HPV DNA was detected by the hybrid capture assay marketed as ViraType Plus by Digene Diagnostics, Beltsville, Md. (15). Briefly, RNA probes for 14 HPV types were added to alkali-denatured specimen DNA, and the probes and DNA were allowed to hybridize under high-stringency conditions. Positive specimens were detected by binding the hybridization reaction to tubes coated with a monoclonal antibody to RNA-DNA hybrids. Bound hybrids were detected by the addition of an alkaline phosphatase-conjugated antibody to the RNA-DNA hybrids and then the addition of LumiPhos and reading in an Optocomp I luminometer (MGM Instruments, Hamden, Conn.). The HPV probes were used as two pools whose compositions were based on the association of each type with genital tract malignancy. Probe group A contained the "low-risk" HPV types 6, 11, 42, 43, and 44, while probe group B contained the "high-risk" HPV types 16, 18, 31, 33, 35, 45, 51, 52, and 56. Positive controls consisted of 1 pg of HPV-11 DNA (for probe group A) or 1 pg of HPV-16 DNA (for probe group B) diluted in 5 µg of HPV-negative DNA, each of which was run in triplicate with each assay. Patient samples were considered positive if the number of relative light units read from the luminometer was greater than the mean of the positive control values. The positive control value had to be  $\geq 1.5$  times the negative control value for the test to be considered valid. Additional positive controls included DNA extracted from an HPV-11-infected human foreskin grown in an athymic mouse (3) (probe A) and linearized HPV-16 DNA added to 5 µg of salmon sperm DNA (probe B).

**Southern blots.** Sample DNA (5 to 10 µg) was digested with *Pst*I for 2 h at 37°C. Following agarose gel electrophoresis, the gel was soaked in 0.2 M HCl for 5 min and rinsed

with distilled water. The DNA was transferred in 0.4 M sodium hydroxide to a nylon HybondN<sup>+</sup> membrane (Amersham) by the method of Southern (21). After the transfer, the nylon was rinsed in 2× SSC (1× SSC is 0.15 M NaCl plus 15 mM sodium citrate) and was allowed to air dry. Prehybridization was performed at 65°C for 16 h in a solution containing 5× SSPÉ (1× SSPE is 0.01 M sodium phosphate, 0.18 M NaCl, and 1 mM EDTA), 0.5% SDS, 5× Denhardt's solution (7), and 50 µg of tRNA per ml. Hybridization conditions were identical to those for prehybridization, with the addition of 5 × 10<sup>6</sup> cpm (per ml) of heat-denatured, <sup>32</sup>P-labelled genomic DNA of HPV types 6b, 11, 16, and 18 purified from the pBR322 vector. The nylon membrane was then washed in decreasing salt concentrations of 6× SSC, 2× SSC, and 0.1× SSC, each with 0.5% SDS at 65°C, and autoradiography was performed.

The sensitivity of the Southern blot method was tested by adding known amounts of linearized, whole genomic HPV DNA to salmon sperm DNA in amounts corresponding to 0.05, 0.1, 0.5, and 2.5 copies per cell. Following electrophoresis, transfer to nylon, and hybridization as described above, autoradiography was performed for 72 h.

**Confirmation of multiple HPV types in condylomata acuminata.** After the initial Southern blot and hybrid capture analysis, three patient samples were analyzed in replicate Southern blots by using a mixture of either HPV type 6 and 11 probes or HPV type 16 and 18 probes under the conditions described above. These three samples were chosen because (i) adequate DNA was available and (ii) the hybrid capture assay showed positivity with both the A and B probes.

**Characterization of HPV-6 subtypes in condylomata acuminata.** Several lesions contained HPV DNAs that produced strong bands in the Southern blot assays relative to the bands produced by the majority of lesions. Representative samples with *Pst*I restriction patterns consistent with known HPV types were chosen for performance of restriction enzyme digestion with *Bam*HI and *Hinc*II. This was done to verify the presence of the suspected HPV-6 subtype. Five micrograms of DNA was used for each digestion. Southern blots were performed as described above.

## RESULTS

**Characterization of patients.** Forty patients (11 males and 29 females) underwent biopsy of typical exophytic condylomata acuminata of the penis, vulva, or perianal areas (Table 1). The patients were selected by the presence of typical exophytic condylomata acuminata and willingness to undergo an excision biopsy. Two male patients (patients 31 and 34) were infected with the human immunodeficiency virus. The female patient population included two with human immunodeficiency virus infection (patients 1 and 40), two renal transplant recipients (patients 22 and 26), one liver transplant recipient (patient 39), and eight who were pregnant (patients 3, 6, 7, 8, 11, 13, 21, and 27). One female patient (patient 4) had a history of vulvar carcinoma, which was removed by vulvectomy, and had a recurrence of typical exophytic condylomata acuminata.

**Histology.** Each of the 40 samples was submitted for routine histopathologic evaluation. Histologic features characteristic of condylomata acuminata were identified in 39 lesions (14). One of the 40 samples (that from patient 28) demonstrated histologic abnormalities of such a minor degree that the diagnosis of condylomata acuminatum could not be made. While all 39 samples demonstrated the char-

TABLE 1. Characteristics of the patients in the study<sup>a</sup>

Patient no.	Sex	No. of HPV copies per cell for probe <sup>b</sup> :		Underlying condition
		A	B	
M	NA	462.90	Neg	NA
1	F	42.80	Neg	HIV
2	F	1.60	Neg	None
3	F	3.47	Neg	PREG
4	F	0.06	1.08	Vulvar carcinoma
5	F	0.38	Neg	None
6	F	0.78	Neg	PREG
7	F	2.10	0.07	PREG
8	F	61.10	Neg	PREG
9	F	1.17	Neg	None
10	F	4.64	Neg	None
11	F	0.62	Neg	PREG
12	F	1.16	Neg	None
13	F	17.89	Neg	PREG
14	F	1.80	0.11	None
15	F	2.27	0.35	None
16	F	0.74	Neg	None
17	F	4.72	Neg	None
18	M	8.84	0.06	None
19	M	5.61	Neg	None
20	M	6.84	Neg	None
21	F	235.88	0.06	PREG
22	F	7.45	1.05	Renal TRP
23	F	183.47	0.08	None
24	F	4.43	1.39	None
25	M	0.81	Neg	None
26	F	0.05	5.00	Renal TRP
27	F	0.72	Neg	PREG
28	F	Neg	Neg	None
29	F	38.81	Neg	None
30	M	5.31	0.53	None
31	M	60.77	0.13	HIV
32	M	10.66	Neg	None
33	M	0.52	Neg	None
34	M	42.39	0.15	HIV
35	M	0.88	Neg	None
36	F	0.90	Neg	None
37	F	0.87	Neg	None
38	M	5.32	Neg	None
39	F	72.61	0.06	Liver TRP
40	F	1.41	Neg	HIV

<sup>a</sup> Abbreviations: NA, not applicable; F, female; M, male; Neg, negative; TRP, transplant; PREG, patient was pregnant at the time the lesion was removed; HIV, human immunodeficiency virus antibody was detected in the patient's serum at or before the time of biopsy.

<sup>b</sup> The calculated number of HPV DNA copies per cell for the A and B probe groups of the hybrid capture assay as defined in the text.

acteristic exophytic papillary pattern under low-power examination, the numbers, sizes, and contours of these papillary processes were variable. Koilocytes were noted in 23 of the 39 samples. Koilocytosis was focal in some samples and more extensive in others. In those samples lacking koilocytosis, other features characteristic of condylomata acuminata such as acanthosis, hyperkeratosis, parakeratosis, and dyskeratosis were noted. Two condylomata acuminata with koilocytosis (removed from patients 22 and 26) demonstrated changes characteristic of a high-grade squamous intraepithelial lesion (moderate dysplasia). In these two cases, nuclear crowding and moderate nuclear atypia characterized by nuclear enlargement and pleomorphism, hyperchromasia, and coarsening of the nuclear chromatin involved approximately two-thirds of the epithelium. HPV-16 was detected in both of these lesions by Southern blotting.

**Hybrid capture assay.** Controls for the hybrid capture assay consisted of HPV-11-infected human foreskin grown in an athymic mouse (3) (probe A) and linearized HPV-16 DNA added to 5 µg of salmon sperm DNA (probe B). For HPV-11-containing DNA from the mouse implant, the signal for the A probe was equivalent to 462.9 copies per cell (Table 1). The B probe was negative when tested with DNA from this sample. For HPV-16, 100 pg, or the equivalent of approximately 5 copies per cell, produced a signal with the B probe that was 88.8 times the positive control signal, corresponding to 4.2 copies per cell. The A probe with this amount of HPV-16 was negative (data not shown). When the equivalent of 20 copies of HPV-16 per cell was used, a signal with the B probe was produced corresponding to 12.6 copies per cell, and the A probe became positive, producing a signal corresponding to 0.1 copy per cell (data not shown). It would appear that crossover with the B probe is minimal, at least for HPV-11, but that crossover occurs with the A probe at approximately 10 viral copies of HPV-16 per cell.

The hybrid capture assay results are shown in Fig. 1. Of the 39 lesions positive by hybrid capture, 25 were positive for the A probe only and 14 hybridized with both the A and the B probes. In 12 of the 14 samples, the A probe signal was stronger than the B probe signal. No condylomata acuminata was positive with the B probe only. The single lesion which was negative by hybrid capture (that from patient 28) did not demonstrate histology consistent with HPV infection.

The intensity of the signal in the hybrid capture assay (relative light units) is directly proportional to the amount of HPV DNA in the sample over a range of concentrations between approximately 1 pg and 1 ng (9a, 15). Nine of the patient samples contained such large concentrations of HPV DNA that they were diluted 1:100 to obtain a reliable concentration estimate. In each case, it was the reaction with the A probe group that fell outside the linear range. No samples detected by the B probe were outside the linear range.

The relative light units were converted to the estimated number of HPV DNA copies per cell by using the conversion of 1 pg of HPV DNA in 5 µg of cellular DNA equals 0.05 HPV copy per cell. These values, ranging from 0.05 to 236 copies per cell for the A probe and 0.05 to 5 copies per cell for the B probe, are shown in Table 1 along with the patients' underlying conditions.

**Southern blots.** Thirty-nine of 40 condylomata acuminata contained detectable HPV DNA by the Southern blot method. HPV-6 subtypes were detected in 28 (72%), HPV-11 was detected in 8 (20.5%), and HPV-16 was detected in 3 (7.5%) of the 39 HPV DNA-containing lesions. HPV-6 subtypes included 27 HPV-6a and 1 HPV-6c *PstI* restriction pattern (Fig. 2). No lesion contained HPV-6b. All detected HPV sequences corresponded to a typical *PstI* restriction pattern for one of the HPV types noted above. Restriction patterns were generally evident on autoradiograms after 48 h; however, for some patterns, 10 days was required for detection.

Condylomata acuminata from the eight pregnant women all contained HPV-6 (seven HPV-6a and one HPV-6c). The three HPV-16-containing lesions were all removed from female patients. Two of these three women were renal transplant recipients (patients 22 and 26) and one was the patient with a history of vulvar carcinoma (patient 4).

The sensitivity analysis showed that the equivalent of 0.1 to 0.5 copy of HPV DNA per cell could be detected after 72 h of autoradiography (data not shown). This level of sensi-

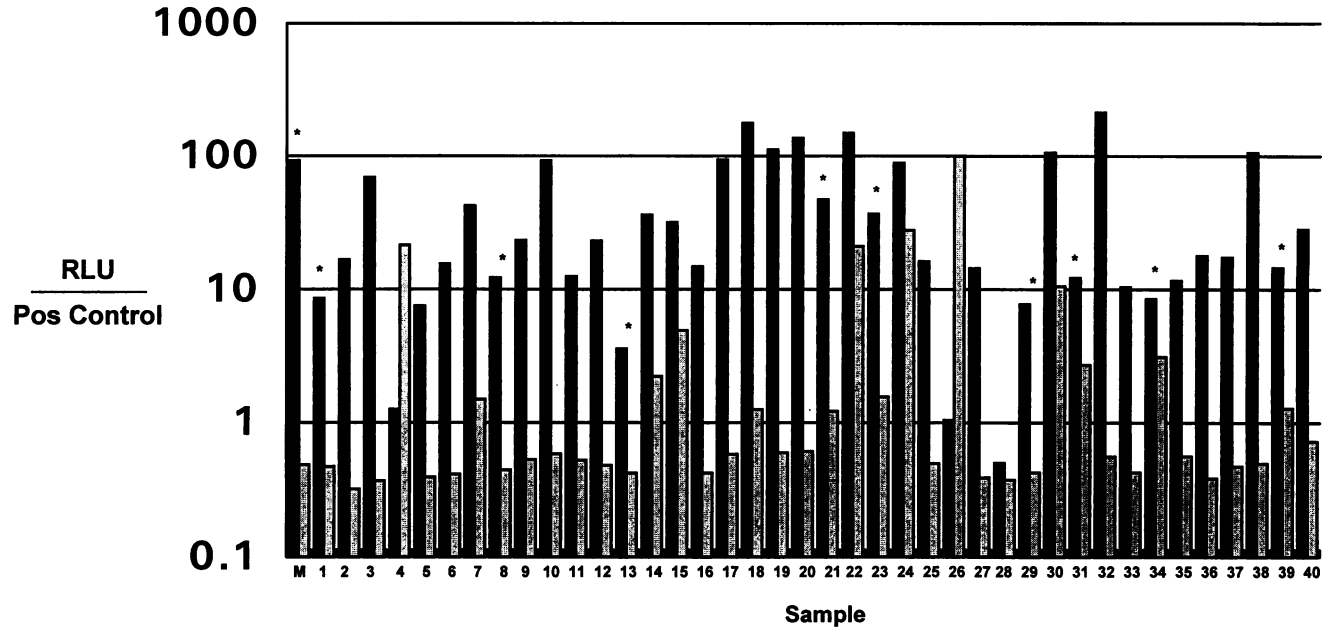


FIG. 1. Results of the hybrid capture assays. The ratios of the signal (in relative light units; RLU) from the patient specimen to that for the positive control (Pos Control) is shown for each sample and for both the A (■) and B (▨) probe groups (see text for probe group compositions). Values of  $\geq 1$  are considered positive. The lane labeled M contains DNA from an experimental HPV-11-infected human foreskin grown in an athymic mouse. Note that nine of the DNA samples were diluted 100-fold before being assayed with the A probe group; these are indicated by asterisks.

tivity is consistent with that described in other published reports (20).

**Comparison of hybrid capture and Southern blot.** Thirty-nine lesions were positive by both hybrid capture and Southern blot analyses. One lesion, which was histologically negative for HPV infection, was negative by both assays. Of the 25 samples positive by hybrid capture for the A probe only, Southern blotting showed HPV-6 in 22 samples and HPV-11 in 3 samples (Table 2). In 14 samples, the hybrid capture assay was positive for both the A and the B probes, with the A probe signal dominant in 12 samples and the B probe signal dominant in 2 samples. In the 12 A probe-dominant samples, Southern blots showed HPV-6 subtypes in 6 samples, HPV-11 in 5 samples, and HPV-16 in 1 sample. For the two B probe-dominant samples, the Southern blot showed HPV-16.

**Multiple HPV types in condylomata acuminata.** Three samples were analyzed in replicate Southern blots. Two clearly showed evidence of more than one HPV type. The first sample was from patient 24. Hybrid capture analysis of DNA from this sample was positive for the A probe (copy number, 4.43) and the B probe (copy number, 1.39). The Southern blot analysis initially showed only an HPV-11 pattern, but replicate blots showed both HPV-11 and HPV-18 patterns (Fig. 3). The second sample was removed from patient 26. Hybrid capture analysis of DNA from this sample was positive for the A probe (copy number, 0.05) and the B probe (copy number, 5.0). The Southern blot analysis initially showed the HPV-16 pattern, and replicate blots showed only the HPV-16 *Pst*I pattern (Fig. 3). The third sample was removed from patient 34. Hybrid capture analysis of DNA from this sample was positive for the A probe (copy number, 42.39) and the B probe (copy number, 0.15). The Southern blot analysis initially showed only an HPV-11

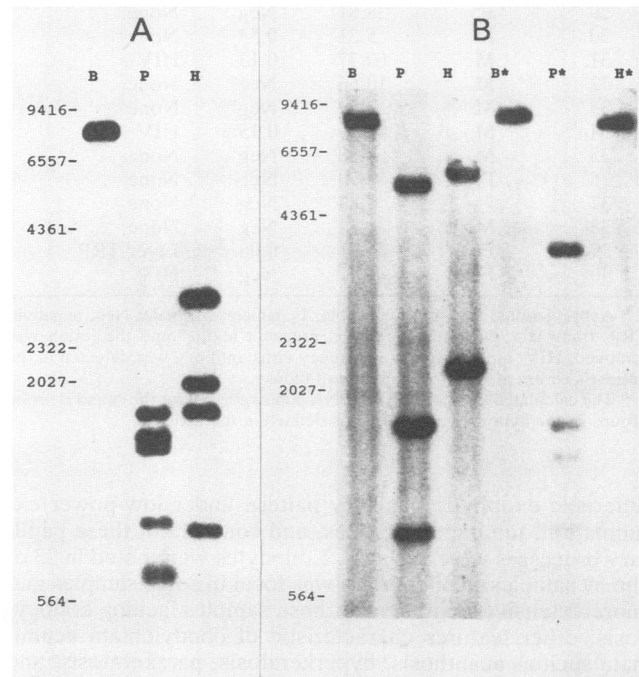


FIG. 2. Southern blots of DNA from condylomata acuminata containing HPV subtypes 6a and 6c and, for comparison, HPV-11. (A) HPV-11 digested with *Bam*HI (B), *Pst*I (P), and *Hinc*II (H). (B) HPV-6c is shown on the left side of the figure, in the first three lanes. The same three restriction endonucleases used in panel A were used in panel B. Note that *Hinc*II cuts HPV-6c twice, producing two fragments. HPV-6a is shown on the right side of panel B, indicated by asterisks beside the three restriction endonucleases. A single *Hinc*II site is present in HPV-6a. Markers on the left are in base pairs.

TABLE 2. Comparison of hybrid capture and Southern blot results for 39 HPV-positive condylomata acuminata<sup>a</sup>

Southern blot result	No. of samples hybrid capture probe positive ( <i>n</i> = 39) <sup>b</sup>			
	A	A/b	B/a	B
Total ( <i>n</i> = 39)	25	12	2	0
HPV-6	22	6		
HPV-11	3	5		
HPV-16		1	2	

<sup>a</sup> Southern blots were performed with whole genomic DNA probes of HPV types 6b, 11, 16, and 18, which were used concurrently. Results of replicate Southern blots are not included.

<sup>b</sup> A, A probe positive only by hybrid capture; A/b, both A and B probe positive by hybrid capture (A probe gives dominant signal); B/a, both A and B probe positive by hybrid capture (B probe gives dominant signal); B, B probe positive only by hybrid capture.

pattern, but replicate blots showed both HPV-11 and HPV-16 patterns (data not shown).

### DISCUSSION

Condylomata acuminata are frequently observed lesions in sexually active individuals. When present on the external genitalia, they are almost always benign lesions, with only rare foci of dysplasia or malignancy being observed. Previous studies, as well as the present study, have demonstrated the presence of HPV type 6 or 11 DNA in the majority of typical exophytic condylomata acuminata of the external genitalia in both sexes.

The HPV types found in the condylomata acuminata in our study were similar to those found by Gissmann et al. (10). We found that HPV-6a is the most common HPV-6 subtype (27 of 28 HPV-6-containing biopsy samples) and, overall, was the most common HPV type (27 of 39) found in biopsies performed in Indianapolis. The second most common type was HPV-11 (8 of 39); this was followed by HPV-16 (3 of 39) and HPV-6c (1 biopsy sample). No biopsy samples contained HPV-6b. Other HPV-6 subtypes have been described in respiratory tract lesions (25), such as HPV types 6e and 6f. We did not detect these types in our condylomata acuminata samples. The HPV-6 subtypes found in genital and respiratory lesions may behave differently with regard to length and abundance of viral transcripts (8, 25).

The Southern blot is considered to be the standard typing method to which other methods are compared (21). The assay is labor-intensive and costly. Results of Southern blots are often difficult to interpret, and agreement between established laboratories is not ideal (2). The assay is sensitive, however, and can detect as little as 0.1 copy of HPV DNA per cell. In addition, a specific type can be determined on the basis of the restriction pattern visualized on autoradiograms. Difficulty may arise when more than one HPV type is present in a lesion, especially if one type is present at a much higher copy number than the other. A very strong HPV-6 signal on an autoradiogram could obscure a weaker signal for another type such as HPV-16. This problem could be corrected, in part, by using individual probes on replicate blots or reprobings the same blot several times with individual probes or groups of probes. Such procedures further increase the work load required for Southern blotting. The replicate Southern blots, using either a mixture of HPV types 6 and 11 or HPV type 16 and 18 whole genomic probes,

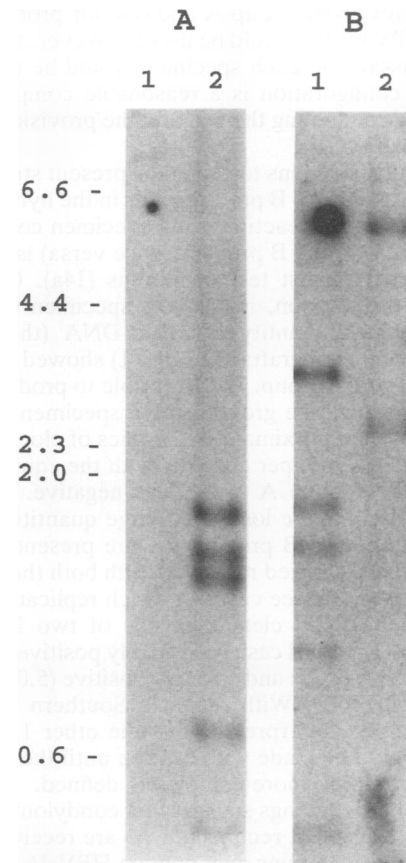


FIG. 3. Southern blot analysis of condylomata acuminata from patients 24 and 26. DNA was digested with *Pst*I, separated by electrophoresis on two identical agarose gels, and transferred to two nylon membranes. One membrane was probed with a mixture of HPV types 6 and 11 (A) and one was probed with a mixture of HPV types 16 and 18 (B). Lanes 1, DNA from patient 26; lanes 2, DNA from patient 24. Markers, in kilobase pairs, are shown on the left.

demonstrated the presence of multiple HPV types in two of the three condylomata acuminata analyzed by the Southern blot method.

The hybrid capture assay is a new method that shows great promise for widespread use in diagnostic laboratories. Because the technique does not use radioisotopes and is conducted much like enzyme-linked immunoassays commonly used in diagnostic laboratories, it could be incorporated into existing laboratories very easily. Hybrid capture is also valuable in that it can detect as little as 0.05 copy of HPV DNA per cell, compared with the 0.1 to 0.5 copy detected by the Southern blot assay. Another advantage is that it can readily detect infections with multiple HPV types, provided that the types are included in the appropriate probe groups, even when the quantities of the different types vary by orders of magnitude. The only method that may be more sensitive than hybrid capture is the polymerase chain reaction. However, there are still a number of technical barriers that have made routine use of the polymerase chain reaction for HPV diagnosis difficult. One limitation of the hybrid capture assay as currently configured is that it does not provide specific typing information and it does not identify specimens infected with multiple virus types within the same probe group. Currently, two assays are performed on each

sample: one for probe group A and one for probe group B. Individual HPV probes could be used; however, the expense of multiple assays on each specimen would be prohibitive. The current configuration is a reasonable compromise between ease of conducting the test and the provision of useful typing information.

A number of specimens tested in the present study reacted with both the A and the B probe groups in the hybrid capture assay. False-positive reactivity of a specimen containing an A group DNA with the B probe (or vice versa) is thought to be minimal under most test conditions (14a). Our results confirm this observation, in that the specimen which contained the largest quantity of HPV DNA (the HPV-11-infected foreskin xenograft; M in Fig. 1) showed no reactivity with the B probe group. We were able to produce a weak signal with the A probe group using a specimen containing the equivalent of approximately 12 copies of cloned HPV-16 DNA (a B group type) per cell, but with the equivalent of 4 copies of HPV 16, the A probe was negative. Therefore, some specificity may be lost when large quantities of HPV DNA, especially of a B probe type, are present. Fourteen patient specimens showed reactivity with both the A and the B probes. In two of three cases in which replicate Southern blots were performed, clear evidence of two HPV types could be seen. The third case was weakly positive (0.05 copy per cell) for the A probe and strongly positive (5.0 copies per cell) for the B probe. With replicate Southern blots, only HPV-16 was seen. Interpretation of the other 11 "mixed" specimens should be made with caution until the limitations of hybrid capture are more completely defined.

Our preliminary findings suggest that condylomata acuminata in organ transplant recipients who are receiving immunosuppressive medications may contain HPV-16 more often than lesions from other patients who are not immunosuppressed. Many women who have condylomata acuminata of the external genitalia also have cervical HPV infection. Therefore, lesions of the external genitalia containing HPV-16 may predict the presence of this HPV type in lesions of the cervix. Renal transplant patients treated with immunosuppressive medications such as glucocorticoids and cyclosporin A have a malignancy rate estimated to be 100 times that of the general population (17). Contributing to this overall excess in malignancy is a 14-fold increase in the incidence of carcinoma in situ of the cervix. The detection of infection with HPVs is also increased in renal transplant patients (19), and the association of cervical HPV infection and the development of cervical malignancy is well established. Progression from intraepithelial neoplasia to invasive carcinoma occurs more frequently and at a more rapid rate in immunosuppressed patients than in the general population (17). Anogenital neoplasms in immunosuppressed patients often occur at relatively young ages, involve multifocal locations, and tend to persist, recur, and progress rapidly, despite presumably adequate therapy.

In summary, the most common HPV type found in condylomata acuminata biopsy samples in the Indianapolis area was HPV-6a. HPV-16 was found in 3 of 40 condylomata acuminata, and 2 of these lesions were removed from renal transplant recipients. The hybrid capture assay proved to be reliable in determining the presence of HPV DNA. The results of the hybrid capture assay were in agreement with those of the Southern blot assay in all cases, although the specific HPV type cannot be determined by the assay as it is currently structured. On the basis of the results of the hybrid capture assay, and as confirmed in two cases by Southern blot analysis, the number of condylomata acuminata con-

taining multiple HPV types appeared to be higher in our study than in previous reports (1, 26). Studies are under way to verify this finding. Further studies are needed to determine the significance of HPV-16 found in exophytic condylomata acuminata, especially in immunosuppressed patients.

#### ACKNOWLEDGMENTS

This study was supported in part by The Burroughs Wellcome Young Investigator Award in Virology from the Infectious Disease Society of America, the Research Advisory Group of the Department of Veterans Affairs, and cooperative agreement AI31494 (4) from the National Institute of Allergy and Infectious Diseases.

We thank Victoria Bell for technical assistance and Attila Lorincz for helpful advice.

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