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Intermethod Variation in Detection of Human Papillomavirus DNA in Cervical Smears

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In order to investigate the reliability of detection of human papillomavirus (HPV) DNA in cervical smears, we have compared the performance of two HPV PCR systems, the CPI/IIG and MY09/11 primer-mediated PCRs and the Hybrid Capture System HPV DNA detection test (hybrid capture assay), in detecting HPV DNA in cervical smears. We also included in our study the MY09/11B PCR plus SHARP (solution hybridization assay for PCR products) Signal System. This SHARP Signal System was recently developed to detect MY09/11B-generated biotinylated PCR products. The detection rate of the hybrid capture assay was lower than those of the CPI/IIG and MY09/11 PCRs and the MY09/11B PCR plus SHARP Signal System. The detection rates of the CPI/IIG PCR and the MY09/11B PCR plus SHARP Signal System were similar and higher than that of the conventional MY09/11 PCR system. The agreement beyond chance of the PCR methods was nearly perfect (kappa value between 0.82 and 0.84). The agreement beyond chance of the hybrid capture assay and the PCR methods was fair to good (kappa value between 0.64 and 0.70). The systems detected HPV DNA in different but overlapping sets of smears. Our results indicate that each of the detection methods alone underestimates the prevalence of HPV.

Cytological analysis of cervical smears is generally used in screening for cervical cancer and premalignant cervical lesions (cervical intraepithelial neoplasia [CIN]) which subsequently can be diagnosed by colposcopy and histopathological examination of cervical biopsies. The recognition that certain human papillomaviruses (HPVs), including HPV type 16 (HPV16) and HPV18, -31, -33, -35, -45, -51, -52, -56, and -58, are underlying causal agents of cervical cancer has raised the expectation that HPV DNA detection could be of value in the identification of women at risk for cervical (pre)malignancies or with concomitant (pre)malignant lesions. A number of epidemiological studies have supported this hypothesis. The prevalence of oncogenic HPVs in cervical cancers as determined by PCR analysis amounts to about 90% (8, 9, 14, 17–19), and the prevalence of oncogenic HPVs in cervical biopsies is strongly correlated with the grade of the CIN lesions. The prevalence rates for the oncogenic HPV types range between 26 and 67% for grade I CIN lesions and 75 to 86% in grade II or III lesions (1, 3, 5, 12). Cuzick and coworkers (6) showed that the detection of high levels of HPV16 DNA in smears by PCR correlated with the presence of a high-grade CIN lesion. Koutsky and coworkers (10) observed that in cytologically normal smears the presence of HPV DNA as determined by the Virapap dot blot hybridization method correlated with the detection of high-grade CIN lesions within 3 years.

PCR-mediated detection of HPV DNA in cervical smears has revealed a low prevalence of oncogenic HPV types (1.5%) in normal smears of women participating in a screening program for cervical cancer, in contrast to high prevalence rates in mild to moderately (41%) or severely (58%) dysplastic smears (18). Higher prevalence rates may be found in cytologically normal smears for selected populations, e.g., women from a gynecological outpatient population (2, 13). Adequate HPV DNA detection methods are essential for the interpretation of epidemiological findings with respect to the role of HPV infections in the etiology of cervical cancer (7). Several HPV DNA detection methods have been described, each of which allows the detection of a spectrum of HPV types. In order to evaluate the variability between some of these methods (intermethod variability), we have applied the Hybrid Capture System HPV DNA detection test (hybrid capture assay) (11), the MY09/11 primer-mediated PCR (2), the CPI/IIG primer-mediated PCR (16), and the MY09/11B PCR in combination with a newly developed nonradioactive detection system (SHARP [solution hybridization assay for PCR products] Signal System) (Digene Diagnostics, Inc., Silver Spring, Md.) to a series of smears with normal or abnormal cytology.

MATERIALS AND METHODS

Collection of cytological and DNA specimens. Cytological cervical smears (n = 206) were obtained from patients attending the outpatient clinic of the Academic Medical Center in Amsterdam, The Netherlands, and four other hospitals which refer their material for cytological examination to the Public Health Laboratory in Enschede, The Netherlands. Epithelial cells from the cervical transformation zone and the endocervical canal were collected with a wooden Ayre spatula, and smears were made. The slides were immediately fixed in 95% ethanol and stained for cytological examination. For HPV DNA detection, cells were collected with a second swab and placed in 1 ml of Virapap transportation medium in Virapap collection tubes (Digene Diagnostics). These cells were kept at +4°C for short-term storage and at −20°C for long-term storage according to the specifications of the Virapap determination protocol.

Cytological classification. The following cytological diagnoses were made by using a slight modification of the Pap procedure as commonly used in the Netherlands: Pap I, normal cells; Pap II, inflammation; Pap IIIA, mild to moderate dysplasia; Pap IIIB, severe dysplasia; Pap IV, carcinoma in situ; and Pap V, (micro-)invasive cancer. For the 206 smears, cytological classification revealed Pap I or Pap II in 87 cases (42%), Pap IIIA in 66 cases (32%), and Pap IIIB to

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V in 53 cases (26%). The laboratory workers performing the HPV DNA detection assays were blinded for the cytological classification.

Hybrid Capture System HPV DNA detection test. HPV DNA detection using the Digene hybrid capture assay was performed explicitly according to the manufacturer's specifications for the hybrid capture assay kit. Briefly, 500 μl of each sample was taken after being thoroughly mixed, 250 μl of denaturation reagent was added, and the samples were incubated at 65°C for 45 min. Then 50 μl of the low-risk (HPV6, -11, -42, -43, and -44) probe mixture (probe A) or 50 μl of the high- and intermediate-risk (HPV16, -18, -31, -33, -35, -45, -51, -52, and -56) probe mixture (probe B) was mixed with 150 μl of the denatured sample, and the mixture was incubated for another 30 min at the same temperature. Subsequently, the mixtures were transferred to the appropriate capture tubes and incubated with the detection reagents. Finally, the extinction values were read in a luminometer and expressed as relative light units (RLU). The positivity or negativity of the samples is established from a ratio, that is, sample RLU/mean RLU of three evaluations of the positive control (10 pg of viral DNA). The sample is positive if the ratio is ≥1 and negative if the ratio is <1.

DNA purification. For detection of DNA by PCR amplification, 100 μl of Virapap transportation medium containing scraped cervical cells was taken after being thoroughly mixed. DNA was extracted once with phenol-chloroform-isoamyl ethanol and precipitated with ethanol. Finally, after the precipitate was washed with 70% ethanol, the precipitated DNA was dissolved in 100 μl of Tris-EDTA and stored at −20°C until use.

CPI/IIG and MY09/11 PCRs and SHARP Signal System. Detection of HPV DNA by PCR amplification was performed with two different primer sets (2, 16). The degenerated consensus primer pairs MY09 and MY11 (MY09/11) (2) and CPI and CPIIG (CPI/IIG) (16) amplify an approximately 450-bp fragment in the L1 open reading frame and a 188-bp fragment in the E1 open reading frame, respectively, of a broad spectrum of genital HPV types. All PCRs were evaluated by two investigators. HPV DNA detection using the CPI [5′TTATCA(T/A)ATG CCCA(T/C)TGTACCAT] and CPIIG primers [5′ATGTTAAT(A/T)(G/C)AG CC(A/T)CCAAAATT] (16) was performed by PCR amplification in a 100-μl PCR mixture consisting of 10 mM-Tris · HCl (pH 8.8), 50 mM KCl, 3.6 mM $MgCl_2$, 0.1 mg of bovine serum albumin per ml, 0.2 mM each deoxynucleoside triphosphate, 75 ng of each primer, and 1 U of *Taq* polymerase (Amplitaq) with 2.5 μl of the DNA sample. Forty step cycles (1 min at 94°C, 1 min at 55°C, and 2 min at 72°C) were performed, and then 10 μl of the amplification product was analyzed on a 2% ethidium bromide-stained gel. These conditions differ from those described previously (16). Subsequently, the DNA was transferred by Southern blotting in 0.4 N NaOH–0.6 M NaCl to a Zetaprobe nylon membrane. The blots were baked, prehybridized in 0.5 M Na phosphate (pH 7.4)–7% sodium dodecyl sulfate (SDS)–1 mM EDTA, and hybridized for 18 h at 55°C to a mixture of ^{32}P-labelled, HPV-specific probes (0.5×10^5 cpm of each probe per ml). The probes were prepared by two PCR cycles using the CPI/IIG primer set and 50 ng each of purified CPI/IIG-mediated PCR amplification products of HPV6, -16, -18, -31, -33, -45, and -51. PCR amplification was done in the presence of 0.2 mM unlabelled dATP, dGTP, and dTTP and 10 μCi (1,000 mCi/mmol) of ^{32}P-labelled dCTP. After the second cycle, 0.2 mM unlabelled dCTP was added, and the reaction was then finished by one final cycle with an elongated extension time of 10 min. After hybridization, the blots were washed three times for 10 min each in 3× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.5% SDS and exposed for 72 h to RX X-ray film and intensifying screens.

The MY09/11 PCR was performed as described by Bauer and coworkers (2). Forty cycles were performed for the detection of HPV DNA by the MY09/11 PCR. For the SHARP Signal System (Digene Diagnostics), the MY11 primer is 5′ biotinylated. PCR amplification was performed in a 50-μl PCR mixture consisting of 12.5 mM Tris · HCl (pH 8.3), 62.5 mM KCl, 2.5 mM $MgCl_2$, 0.2 mM each deoxynucleoside triphosphate, 25 pmol of each primer, and 1 U of *Taq* polymerase (Amplitaq). Forty step cycles (1 min at 94°C, 2 min at 55°C, and 3 min at 72°C) were performed. Amplimers were detected exactly as described by the manufacturer. Briefly, a 5-μl sample of PCR mixture was denatured in base and hybridized to two different sets of unlabelled RNA probes. Set A contains probes for the nononcogenic HPV types (HPV6, -11, -42, -43, and -44. Set B contains the oncogenic HPV probes (HPV16, -18, -31, -33, -35, -39, -45, -51, -52, -56, and -58). The hybridization mixture was transferred to a streptavidin-coated microplate on which the RNA-DNA hybrids were captured. These hybrids were subsequently detected by an alkaline phosphatase-labelled antibody directed against RNA-DNA hybrids. Colorimetric measurements were obtained by using *para*-nitrophenylphenol as a substrate in a microtiter reader. Appropriate negative and positive controls were included as prescribed by the manufacturer.

To determine whether samples were adequate for HPV detection by PCR amplification, PCR amplification of a 172-bp fragment of the β-globin gene was performed for each sample, using primers glo-1 (5′ACACAACTGTGTTCACT ACC) and glo-3 (5′TCTATTGGTCTCCTTAAACC). Forty PCR cycles were performed in 10 mM Tris · HCl (pH 8.3)–50 mM KCl–1 mM $MgCl_2$–10 μg of albumin per ml. All samples were positive with these primers. In between the patient samples, negative controls (water only) were used throughout the extraction procedure. The results of PCR amplification of these samples using each of the primer pairs remained negative.

HPV typing. The HPV types of samples positive in the CPI/IIG-mediated PCR were determined as previously described (15) by direct sequence analysis of the purified 188-bp PCR product using the 3′ primer (CPI) as a sequencing primer and comparison of the sequence with the sequences of HPV6, -11, -16, -18, -31, -33, -35, -39, -42, -45, -51, and -58. PCR products were concentrated and purified prior to sequence analysis by phenol-chloroform extraction, ethanol precipitation, and agarose gel electrophoresis. The appropriate bands were extracted from the agarose gel according to a modification of the procedure described by Boom and coworkers (4). Briefly, agarose gel slices were dissolved in 0.9 ml of 5.25 M guanidinium isothiocyanate–50 mM Tris · HCl (pH 6.4)–20 mM EDTA–1 mg of casein per ml for 40 min at 37°C in the presence of 5 μl of activated silica. After brief centrifugation for 15 s, the nucleic acid-silica complex was washed twice with 5.25 M guanidinium isothiocyanate in 50 mM Tris · HCl (pH 6.4), twice with 70% ethanol, and once with acetone, and after brief drying of the silica at 56°C, the DNA was eluted in 15 μl of Tris-EDTA at 56°C.

Statistical evaluation. The intermethod variation between the tests was determined by calculating kappa values with 95% confidence intervals for each combination of single tests. Kappa values express the agreement beyond chance. Generally, a kappa value of >0.80 represents almost perfect agreement beyond chance. Values below 0.40 represent slight agreement, and values between 0.40 and 0.80 represent fair to good agreement. The relation between Pap classification and viral DNA level was analyzed by an analysis of variance procedure.

TABLE 1. Detection of HPV DNA by four methods

Method	No. (%) of smears HPV DNA positive			
	All smears (n = 206)	Pap I and II (n = 87)	Pap IIIA (n = 66)	Pap IIIB to V (n = 53)
CPI/IIG PCR	106 (51)	10 (11)	47 (71)	49 (92)
MY09/11 PCR	96 (47)	6 (7)	41 (62)	49 (92)
MY09/11B (SHARP)	107 (52)	12 (14)	45 (68)	50 (94)
Hybrid capture	80 (39)	9 (10)	31 (47)	40 (75)

RESULTS

HPV prevalence. HPV positivity as determined by PCR ranged from 47% for the MY09/11 PCR to 51% for the CPI/IIG PCR and 52% for the MY09/11B (SHARP Signal System) PCR (Table 1).

With a total of 80 positive smears (39%), the detection rate of the hybrid capture assay (probes A and B) was lower than that of the PCRs. Whereas within the present study group the highest percentage of positive smears (52%; 107 smears) was observed with the MY09/11B (SHARP Signal System) PCR, a total of 57% (118 smears) was judged positive by taking into account the results of all HPV DNA detection methods. The four methods agreed (results all positive or negative) for 78% of all smears (160 of 206) and for 61% of the HPV-positive smears (72 of 118) (Table 2).

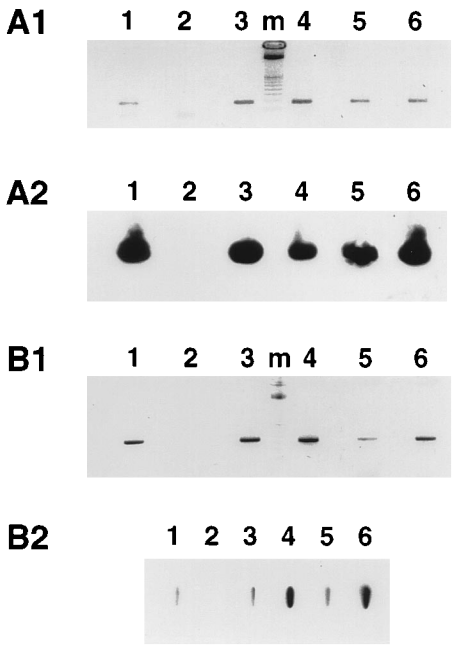
The detection rates of the four HPV DNA detection meth-

TABLE 2. Comparison of four HPV DNA detection methods

Result by the following method:				No. of smears		
Hybrid capture	CPI/IIG	MY09/11	MY09/11B (SHARP)	Pap I and II (n = 87)	Pap IIIA (n = 66)	Pap IIIB to V (n = 53)
−	−	−	−	69	16	3
+	−	−	−	1	1	0
−	+	−	−	2	1	0
−	−	+	−	1	0	0
−	−	−	+	4	2	0
+	+	−	−	2	1	0
−	+	+	−	0	2	0
−	−	+	+	1	0	1
+	−	−	+	1	0	0
+	+	−	+	1	1	0
−	+	−	+	1	3	1
−	+	+	+	0	11	8
+	+	+	+	4	28	40

ods were also calculated for the different Pap classes of the smears. The prevalence of HPV DNA as detected by the four methods ranged between 7 and 14% for the group of normal smears (Pap I and II), between 47 and 71% for the group of Pap IIIA smears, and between 75 and 94% for the group of smears containing severely dysplastic or cancerous cells (Pap IIIB to V) (Table 1). The four methods were more consistent in detecting HPV DNA in the high-Pap class smears than in the low-Pap class smears or in the normal smears. Of the 50 HPV-positive group Pap IIIB to V smears, 40 were positive by all four methods (80% agreement), and no smear was positive by only one of the methods. For the Pap IIIA group, only 28 of the 50 HPV-positive smears (56%) were positive by all methods and 4 (8%) were positive by just one of the methods, and for the normal smears these figures were 4 (22% agreement) and 8 (44%), respectively, of 18 HPV-positive smears (Table 2). Analysis of the smears with the hybrid capture assay was done using both probes A and B (see Materials and Methods). Of the 206 smears, 13 (6%) were positive with probe A and 72 (35%) were positive with probe B. Of the 13 smears positive with probe A, 6 were also positive with probe B (and also positive with SHARP probe B). These smears contained HPV16 (three smears), HPV18 (one smear), and HPV33 (one smear). The HPV type of the sixth smear positive for both probes of the hybrid capture assay could not be determined, since the CPI/IIG PCR was negative and HPV typing was performed by sequence analysis of the CPI/IIG PCR product. Two of the seven smears positive with probe A and negative with probe B contained HPV6 or a closely related HPV type, and two contained an unknown HPV type (HPVX). The HPV type of the other three smears could not be determined, as the CPI/IIG PCR result was negative. Only two of the seven hybrid capture probe A-positive smears appeared also to be positive with probe A of the SHARP Signal System. These two smears contained HPV6 and HPVX. Three of these seven smears were positive only with hybrid capture probe A and not with probes A and B of the SHARP Signal System. One of these three smears contained HPVX, and for the other two smears the HPV type could not be determined. The last two of these seven Viratype probe A-positive smears were positive with probe B of the SHARP Signal System and contained HPV6 (one smear) and an HPV type which could not be determined. In conclusion, only 2 of the 13 hybrid capture probe A-positive smears contained HPV6, 5 contained an oncogenic HPV type, 2 contained HPVX, and 4 smears contained an HPV type which could not be determined. Therefore, the results obtained with the two probes in our study were combined for further analysis. Only two smears were positive with only probe A of the SHARP Signal System. One of these smears contained HPV6 DNA and was also positive with probe A of the hybrid capture assay. The other smear was negative as determined by the CPI/IIG PCR as well as the hybrid capture assay. The other nine smears positive with probe A were also positive with probe B of the SHARP Signal System. Five of these smears appeared to be positive with probe B of the hybrid capture assay. Four of these five smears contained HPV31, and one contained HPV16. For further analysis, the results of the hybridizations with probes A and B of the SHARP Signal System were combined.

Generally, of the two PCR-based HPV detection methods, the MY09/11 primer set-mediated PCR described by Bauer and coworkers (2) with analysis on an ethidium bromide-stained gel gave the signals with the highest intensity (Fig. 1). For the MY09/11 PCR, 90 of 96 positive results (94%) were detected after inspection of the gel (data not shown). After amplification by the CPI/IIG primer-mediated PCR, all but



C

Smear	1	2	3	4	5	6
Hybrid capture						
probe A	0.8	2.4	0.6	0.4	0.5	0.4
probe B	15.7	0.4	33.3	0.3	5.5	6.4
MY09/11B PCR (Sharp)						
probe A	0.003	0.003	0.006	0.008	0.005	0.014
probe B	0.398	1.291	1.435	1.558	0.991	1.245
HPV type	16	n.i.	16	16	33	16

FIG. 1. Detection of HPV DNA in cervical smears by four methods. The results of the CPI/IIG PCR (A), the MY09/11 PCR (B), and the hybrid capture assay and MY09/11B PCR plus SHARP Signal System (C) for six cervical smears are shown. The results of the HPV type detected, as determined by direct sequence analysis of the CPI/IIG PCR product, are also given (C). The results of analysis of the PCR products by ethidium bromide staining (A1 and B1) and by hybridization (A2 and B2) are presented. Hybrid capture assay results are ratios (sample RLU/mean RLU of three positive controls); MY09/11B PCR values are extinctions (410 nM). n.i., not identified.

one of the positive samples were readily detected by viewing the ethidium bromide-stained gel. In some instances, bands of low intensity with correct molecular weights which hybridized poorly were seen on ethidium bromide-stained gels. In each of these cases, direct sequence analysis revealed the presence of HPV, and such samples were counted positive (e.g., Fig. 1, B1, lane 5). Doubtful results were obtained for a small number of samples with all methods. The interpretation of the HPV status of these samples did not account for the discrepancies observed between the various methods.

Variability in HPV DNA positivity of cervical smears determined by four HPV DNA detection methods. The differences between the various methods in detecting HPV DNA in cervical smears, as already indicated by the different percentages of smears found positive by each of the methods, were further illustrated by pairwise comparisons of the methods (Table 3). The kappa values ranged from 0.64 for the hybrid capture assay and the MY09/11B (SHARP) method to 0.84 for the MY09/11 PCR and the MY09/11B (SHARP) method.

HPV typing. The HPV types of most of the samples positive by the CPI/IIG PCR could be determined by direct sequence

TABLE 3. Intermethod variation of detection of HPV DNA in cervical smears

Methods	Kappa value (95% confidence interval) all smears (n = 206)
Hybrid capture and MY09/11	0.66 (0.56–0.76)
Hybrid capture and MY09/11B (SHARP)	0.64 (0.54–0.75)
Hybrid capture and CPI/IIG	0.70 (0.60–0.79)
CPI/IIG and MY09/11B (SHARP)	0.83 (0.76–0.91)
CPI/IIG and MY09/11	0.82 (0.74–0.90)
MY09/11 and MY09/11B (SHARP)	0.84 (0.76–0.91)

analysis of the PCR products (15). Almost all of the HPV-positive samples contained oncogenic types (Table 4). In only two samples (Pap IIIA smears) was a nononcogenic HPV type (HPV6) detected. Smears judged positive by the CPI/IIG PCR but negative by other methods contained known high- or intermediate-risk oncogenic HPV types in almost all samples. Smears detected by the CPI/IIG PCR but negative by the hybrid capture method were samples containing HPV16 (five smears), -18 (three smears), -31 (5 smears), -39 (one smear), -45 (two smears), and -58 (two smears) or an unknown HPV type (five smears) and five samples for which the HPV type was not determined. Only two HPV16 samples, one HPV18 sample, one HPV35 sample, and one HPV51 sample and two smears with unknown types were missed by the MY09/11B PCR (SHARP Signal System). Smears with HPV detected by the CPI/IIG PCR but negative by the MY09/11 PCR were samples containing HPV16 (two samples) or HPV18, -31, -39, -45, or -51 (one sample each) or an unknown HPV type (two samples) and two samples for which the HPV type was not determined. The HPV types of the MY09/11 PCR-positive and CPI/IIG PCR-negative samples could not be identified, since typing was performed by sequence analysis of the CPI/IIG PCR amplimers. This result indicates that the different PCR-based methods differ in their rates of detection of most known oncogenic HPV types. This is also true for the hybrid capture assay. In addition, this system lacks probes for HPV39 and HPV58. As a result, one of the two samples containing HPV39 and two of the three samples which were HPV58 positive in the CPI/IIG PCR were not detected with this system. The MY09/11B SHARP Signal System probe B contained these two types, so no HPV39 or HPV58 was missed by that system.

Pap class and the viral DNA level. The hybrid capture assay is a semiquantitative method. In accordance with a previous report, the mean RLU ratio obtained by this method was much lower for the normal smears than for the abnormal smears (Table 5). One-way analysis of variance comparing the four groups (Pap I and II, Pap IIIA, Pap IIIB, and Pap IV and V) showed a significant difference between them (F = 3.42; P = 0.022).

TABLE 4. Pap class and HPV types detected by the CPI/IIG PCR

Pap class(es)	No. of smears with the indicated HPV type											
	ND[a]	6	16	18	31	33	35	39	45	51	58	X
I and II	1	0	3	2	1	1	0	0	0	1	0	1
IIIA	2	2	13	4	7	2	3	2	1	1	2	7
IIIB to V	1	0	25	3	7	3	2	0	0	1	2	4
All	4	2	41	9	15	6	5	2	1	3	0	12

[a] ND, not determined.

TABLE 5. Viral DNA level and Pap grade

Pap class(es)	Mean RLU ratio[a]	SD
I and II	4.8	4.9
IIIA	36.5	62
IIIB	38.5	42.5
IV and V	19.2	23.1

[a] See Materials and Methods.

DISCUSSION

The results for our selected study group indicate that the agreement (beyond chance) of the PCR methods is nearly perfect (kappa values range between 0.82 and 0.84). Our data generally show a fair to good agreement (beyond chance) of the hybrid capture assay with the PCR-based methods. The agreement of the hybrid capture assay with the PCR methods is lower than the agreement between the different PCR methods. These results indicate that the observed intermethod variability is at least partly due to an aberrant detection rate or specificity of the hybrid capture assay. The analysis also showed that each of the methods detected HPV DNA in smears found negative by the other methods. Also, the most sensitive method (MY09/11B PCR plus SHARP Signal System) missed samples which were found positive by the least sensitive method (Viratype) (Table 2). Notably, however, the MY09/11B PCR plus SHARP Signal System did not miss any severely dysplastic or cancerous samples (Pap IIIB to V) which were positive by any method (Table 6). The MY09/11 and CPI/IIG PCRs missed only one of these samples. For the lower Pap classes, the PCR-based methods failed to detect all samples judged positive by any method (Table 6).

The results also indicate that each of the HPV DNA detection methods underestimates the actual prevalence of HPV infections. The underestimation may well be significant, as in the present study group, only between 68 and 91% of all 118 HPV-positive smears were found positive by the individual assays (Table 6). For the separate Pap classes, these values are between 33 and 67% for the Pap I and II smears, between 62 and 94% for the Pap IIIA smears, and between 80 and 100% for the group of Pap IIIB, IV, and V smears. The performance of each of these HPV DNA detection methods is different when applied for the detection of genital HPV DNA, especially in cervical smears with low grades of dysplasia or normal cells. These differences appear to be the result of various detection rates for high-risk genital HPV types. Presumably, the relatively high discrepancy between the methods in the detection of HPV DNA in Pap I and II smears is related to the lower levels of HPV DNA in these smears (Table 5).

The performance of the CPI/IIG PCR as well as the MY09/11B (SHARP Signal System) PCR may overcome the problem

TABLE 6. HPV detection rate expressed as a percentage of smears found positive by any method

Method	No. of positive smears (%) of the indicated Pap grade[a]			
	I and II (n = 18)	IIIA (n = 50)	IIIB to V (n = 50)	Any (n = 118)
Hybrid capture (A and B)	9 (50)	31 (62)	40 (80)	80 (68)
CPI/IIG PCR	10 (56)	47 (94)	49 (98)	106 (90)
MY09/11 PCR	6 (33)	41 (82)	49 (98)	96 (81)
MY09/11B PCR (SHARP)	12 (67)	45 (90)	50 (100)	107 (91)

[a] n, number positive by any method.

of underestimation, since in these tests the number of samples scored positive was raised to 115 of 118 (97%) HPV-positive smears, including 50 of 50 (100%) of the Pap IIIB to V smears and 49 of 50 (98%) Pap IIIA smears.

As mentioned in Results, only two smears were HPV DNA positive with only probe A of the MY09/11B PCR plus SHARP Signal System. All other probe A-positive samples ($n = 9$) were also probe B positive, and four of these smears contained HPV31. This result suggests that HPV31 DNA cross-hybridized with probe A of the SHARP Signal System or that frequent double infections of HPV31 and an HPV type hybridizing with probe A occur. This phenomenon needs further attention.

Execution of each of the PCR detection assays is complicated and time-consuming. The CPI/IIG PCR includes amplification by 40 cycles, ethidium bromide-stained-gel electrophoresis, Southern blotting, hybridization, and typing by sequence analysis. The MY09/11 PCR, according to the original protocol, differs from the CPI/IIG PCR in that the HPV status of the samples is scored by hybridization of dot blots and the PCR products are not analyzed by gel electrophoresis. Also, for typing purposes, the blots are sequentially hybridized with type-specific probes. The latter step was not performed in our study. For both of these PCRs, the number of smears found positive was only marginally increased by hybridization compared with the number found by visual inspection of the ethidium bromide-stained agarose gel. Also, in the great majority of the samples, the typing analysis showed the presence of known oncogenic HPV types. One thus might consider limiting the analysis to PCR amplification and ethidium bromide-stained-gel electrophoresis of the PCR products.

The hybrid capture assay (11) has been designed for the rapid detection of genital HPV types in a clinical setting. The method allows the identification of HPV-positive smears within 1 day and is not subject to product contamination, as is the PCR. Analysis with both probes (A and B) in our study revealed that only 7 of the 206 smears were positive with only probe A and 6 smears were positive with probe A as well as probe B. Five of these smears contained oncogenic HPV types and hybridized exclusively with probe B of the SHARP Signal System. We think that it is conceivable that these samples are false positive with probe A of the hybrid capture assay. The detection rate of the hybrid capture assay is lower than that of PCR amplification, and some types may be missed altogether because of the composition of the probe mixtures. Therefore, probes for HPV39 and HPV58 will be added to this system (11a).

The MY09/11B PCR plus SHARP Signal System is a versatile nonradioactive detection method. An important advantage of this method is that a colorimetric signal which can be read in a conventional microplate reader is developed. By taking into account the controls, an objective cutoff value is determined, so that interpretation of the results is more objective than that of the other PCR readout systems. Also, because of its relatively good detection rate, this method should be evaluated in a clinical study for its usefulness as a diagnostic method. Carefully designed technology assessment studies, such as studies to define the interlaboratory variability, are also very important. Clinical applications of HPV DNA detection could be the identification of patients with ambiguous cytological smears (Pap IIIA) or with false-negative smears, who are at risk for CIN II/III or cervical cancer. As our results showed variation between the performances of the methods, this suggests that the clinical and epidemiological utilities of the methods will be different. To evaluate whether one method or a combination of these methods could complement cytology in the screening for cervical cancer and premalignancies, studies will be required to determine the sensitivities and specificities of the different methods for the detection of HPV DNA.

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