

# Factors associated with clinical and sub-clinical anal human papillomavirus infection in homosexual men

C L H Law, M Qassim, C H Thompson, B R Rose, J Grace, B J Morris, Y E Cossart

## Abstract

**Objectives**—(i) to determine the relative sensitivities of clinical examination, cytology and HPV DNA hybridisation for the detection of anal human papillomavirus infection; and (ii) to examine various factors which may influence presentation of anal human papillomavirus infection in homosexual men.

**Methods and Results**—112 unselected homosexual men attending a Sydney STD clinic for routine screening underwent a complete anogenital and physical examination, during which blood samples (for haematological, serological and immunological investigations), rectal swabs (for culture of anal pathogens) and anal scrapes of the dentate line (for cytology and HPV DNA hybridisation) were collected. Papanicolaou-stained anal smears were examined for cytological abnormalities, including those indicative of HPV infection or anal intraepithelial neoplasia (AIN). HPV DNA was detected by high stringency dot hybridisations using radiolabelled HPV 6, 11, 16 and 18 DNA probes. Visible anal condylomata, situated either externally or in the anal canal, were present in 26% of these men; 46% had cytological evidence of HPV infection, and 19% of the smears showed evidence of mild to moderate dysplastic changes (AIN I-II). Detectable HPV DNA was present in 40% of the anal scrapes. By combining these results, a total of 73 men (65%) were found to have at least one of the indicators of HPV infection. These data, together with that relating to HIV antibody, immune status and past or present infection with other STDs, was correlated with information obtained from a questionnaire administered to the patients at the time of their clinical examination.

**Conclusions**—In this study cytology was found to be slightly more sensitive than HPV DNA dot hybridisation for the detection of HPV infection in the anal canal, providing the full range of HPV-associated cytological changes were accepted as a basis for diagnosis. Clinical anal lesions were more likely to be detected in young men, men who had symptomatic HIV infection and those with a history of past anal wart infection. The latter group also had a higher incidence of cytologically apparent HPV infection in their anal smears. There was a significant association between the detection of HPV 16/18 and the presence of anal dysplasia, but there were no significant correlations between HPV infection or anal dysplasia and HIV antibody, immune function status, sexual practices or history of other STDs.

## Introduction

The increase in the number of reports of anal intraepithelial neoplasia (AIN), carcinoma-in-situ and squamous cell carcinoma in association with anal condylomata in homosexual men<sup>1</sup> indicates a need for better understanding of the prevalence of anal HPV infection in this group and the risk factors associated with neoplastic change. In 1971 Oriel observed that anal condylomata acuminata were more common than penile lesions among homosexual men,<sup>2</sup> but the prevalence of non-condylomatous lesions of the anal region was not fully understood until the introduction of cytological screening of anal smears.<sup>3</sup> Since then, the cytological features of HPV infection in the anal region have been well described, as have the features of AIN.<sup>4,6</sup> The introduction of DNA hybridisation techniques in the detection of HPV DNA has improved our understanding of the broader spectrum of HPV involvement in the lesions of the anal region. HPV types 6 and 11 have been detected in the majority of anal condylomas,<sup>7,8</sup> whereas type 16 has been detected more commonly in anal squamous cell carcinomas.<sup>9,10</sup> Early epidemiological studies of anal cancer defined American men who were young, single and never married as having an increased risk of anorectal carcinomas,<sup>11,12</sup> but a direct association of these cancers with homosexuality and receptive anal intercourse was not demonstrated until recently.<sup>13</sup> Daling

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STD Centre, Sydney Hospital  
C L H Law, M Qassim  
Department of Anatomical Pathology,  
Royal Prince Alfred Hospital  
J Grace  
Department of Infectious Diseases,  
The University of Sydney  
C H Thompson, B R Rose, B J Morris, Y E Cossart

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*et al*, in a study of the risk factors for anal cancer, noted that a history of receptive anal intercourse was strongly associated with the occurrence of anal cancer in men, and that current cigarette smoking and a history of genital warts were additional risk factors for anal cancer in both men and women.<sup>14</sup>

In this report we describe investigations carried out to elucidate the epidemiology of anal HPV infection and AIN in a group of 112 homosexual men. Our aims were to determine firstly the relative sensitivity of the different methods (clinical diagnosis, cytology and molecular hybridisation) utilised for the detection of anal HPV infection, in order to provide valid end-points for prevalence data and for the study of risk factors associated with the different presentations of HPV infection in this group. Our second objective was to investigate various possible risk factors—such as previous anal HPV infection, sexual practices, cigarette smoking, immune status and infection with the human immunodeficiency virus (HIV)—which might correlate with the presence of anal HPV infection or AIN.

## Materials and methods

### *Patients and specimen collection*

This project was approved by the Ethics Committee of Sydney Hospital: informed consent was obtained from the 112 participants prior to entry into the study. These men were homosexuals who had consecutively presented to one of us (CL) for routine screening for STDs and HIV, and were unselected for HPV infection. Each of these subjects completed a questionnaire and underwent a full physical examination by the same investigator, at which time blood specimens were collected for full blood counts, immune function tests and serological screening for HIV antibody and treponemal serology (VDRL and TPHA tests). Rectal swabs were directly inoculated on to modified New York City agar for culture of *Neisseria gonorrhoeae*.<sup>15</sup> For identification of *Chlamydia trachomatis* and *Herpes simplex* virus rectal swabs were placed into transport medium and sent to a reference laboratory for culture.<sup>16 17</sup>

Specimens for cytology were collected by scraping the squamo-columnar junction at the dentate line of the anal canal with the rounded end of a wooden spatula. A second identical specimen for HPV DNA hybridisation was then collected. These two specimens were taken under direct vision via an anoscope illuminated by a Heine light source. The presence of condylomata, or any other abnormal clinical findings, were recorded, but the personnel involved in the cytological and DNA hybridisation investigations were blinded to the clinical findings.

### *Blood counts and immune function tests*

Total and differential white cell counts were per-

formed using a Coulter counter on blood collected in EDTA. Heparinised blood was used for estimation of T cell subsets within 5 hours of collection. Briefly, 50  $\mu$ l of blood was lysed, washed once, reacted with 25  $\mu$ l of fluorescein-conjugated monoclonal antibody (Leu 4 and Leu 3a/2a for total T cells and helper/suppressor T cells respectively; Becton and Dickinson), washed with phosphate buffered saline pH 7.4 (PBS), fixed in 2% paraformaldehyde in PBS, then read in a flow cytometer (Becton and Dickinson FACS 440).

### *HIV antibody tests*

Sera were examined for HIV antibody by ELISA (Abbott Recombinant HIV-EIA, Abbott Laboratories and Wellcozyme anti-HIV III EIA, Wellcome Diagnostics). Positive antibody tests were confirmed by Western Blot assays (DuPont Australia Ltd).<sup>18</sup>

### *Cytological examination of anal smears*

The cellular material collected from the anal canal was spread on a glass slide and immediately fixed with spray fixative (Cytology Fixative, NEACO). In the cytology laboratory it was stained by the routine progressive Papanicolaou stain.<sup>19</sup> The anal smears were routinely screened by a technologist and then reviewed and reported by a cytopathologist (JG). The presence of endocervical cells in cervical smears has been associated with higher detection rates of cytological abnormalities,<sup>20</sup> and this characteristic is routinely used in our laboratory as an indicator that the squamo-columnar junction of the cervix has been adequately sampled. Similarly, in the anal smears the presence of columnar cells was used as an indicator of adequate sampling of the dentate line (squamo-columnar junction) of the anal canal.

The major cytological criterion used to diagnose HPV infection was koilocytosis, as this is considered to be the most reliable cytologic feature.<sup>21 22</sup> Binucleation, nuclear atypia (geometrism), parakeratosis, amphophilia and orangeophilia were also reported and in the absence of koilocytes at least three of these minor features were required to be present in a smear for a diagnosis of HPV infection to be made.<sup>6</sup> Anal dysplasia (AIN) was classified as mild, moderate or severe (AIN I, II or III) according to criteria that have been well described.<sup>23</sup> In those cases where dysplasia and HPV infection were present in the same specimen both were reported.

### *HPV DNA studies*

These were performed essentially as described previously.<sup>24</sup> Briefly, the spatulas on which the anal scrapes had been collected were broken off into tubes containing 1.5 ml of Tris-EDTA collection buffer, and stored for a maximum of 72 hours at 4°C before processing. The cells were dislodged from the

spatulas by vigorous pipetting with buffer, then pelleted (12 000 *g* for 12 s), washed in collection buffer, recentrifuged, and resuspended in 80  $\mu$ l of collection buffer. Simultaneous cell lysis and DNA denaturation were achieved by treating the specimens with 0.5 M NaOH at 95°C for 10 min. The lysates were then transferred to duplicate nylon membranes (Gene Screen Plus, NEN Dupont) with a Biodot<sup>TM</sup> apparatus (Biorad). The nylon membranes were probed using radiolabelled HPV 6/11 and 16/18 DNA probes under conditions of high stringency.<sup>24</sup> HPV DNA standards (125 pg to 0.125 pg of HPV 6, 11, 16 and 18) and appropriate negative and positive controls were routinely included on each membrane. The results were interpreted by a single observer (BR): specimens that produced autoradiographic signals of intensity greater than the 0.125 pg HPV DNA were interpreted as positive; signals less than this standard were regarded as negative.

#### Statistical analyses

The chi square test with Yates' correction and Fisher's exact test were used to determine the statistical significance of association between the different criteria of HPV infection and the variables of interest (age, sexual practices, smoking history, past history of anogenital warts, history of STDs, HIV status and T cell subset values). For continuous variables, the mean value was used as a cut-off point to categorise them for purposes of comparison. A multiple logistic regression model was used to confirm the statistical association between a particular variable and HPV infection, controlling the other independent variables mentioned above.

## Results

#### Characteristics of the study population

The mean age of this group of 112 homosexual men was 34 years (range 18–68). The mean duration of homosexual activity was 15 years (range 2–40 years), and the median of the number of sexual partners over the subjects' life-time was 104 (range 3–6400). One hundred and four (93%) of the subjects admitted to receptive anal intercourse. Smoking history was available from only 69 subjects: 30/69 (44%) had never smoked; 12/69 (17%) had smoked in the past but had stopped smoking; and 27/69 (39%) were current smokers. Because of the lack of data regarding this factor it was excluded from further analysis. The majority of men (89%) reported at least one previous episode of STD (other than HPV) infection. Fifty six per cent (63/112) reported having had anal warts at some stage in the past, including anal warts that had responded to treatment and never recurred, or anal warts that resolved but then later recurred. In contrast, only 13% (15/112) of these men had a history of penile warts.

#### HIV status and immune function tests

Forty per cent (45/112) were positive for HIV antibody. Clinically, only one patient presented with overt AIDS, but 15 men presented with PGL (persistent generalised lymphadenopathy), and one with ARC (AIDS-related complex); the remainder were healthy and asymptomatic for HIV infection. The T cell helper/suppressor (CD4+/CD8+) ratio was within the normal range (1.2–4.1) in 55/112 (49%) of patients, while in 51% the ratio was below the normal range. The majority (38/57, 67%) of patients with subnormal ratios were also positive for HIV antibody while six patients who were positive for HIV antibody had CD4+/CD8+ ratios within normal limits. The absolute values of T helper (CD4+) cells were within normal range (360–1320  $\times 10^9/l$ ) in the majority (85%) of cases, but 17% and 10% of patients, respectively, had values above or below these limits.

#### Detection of ano-genital pathogens

Nine (8%) of the men had positive cultures for *Herpes simplex* types 1 or 2. *Neisseria gonorrhoeae* was cultured from only one patient, and only two patients returned a positive result for *Chlamydia trachomatis*. Treponemal serology was reactive in a high percentage of the men: seven patients had a reactive VDRL test; while 31 were positive by TPHA, indicating a high rate of past (or present) exposure to a treponemal infection.

#### Detection of HPV infection

Twenty six per cent (29/112) of the men had visible perianal and/or internal anal condylomata at the time of examination. Ten patients had external anal warts only, six had internally sited warts in the anal canal

Table 1 Cytological diagnosis of anal smears correlated with the results of HPV DNA probing on the corresponding anal scrapes

Cytological diagnosis	N	HPV DNA probing			
		-ve	6/11 only	16/18 only	6/11 and 16/18
Normal	39	28	9	1	—
Inflammatory	8	5	—	1	2
Para/hyperkeratosis only	13	11	1	1	—
HPV infection*	31	14	7	5	5
HPV infection* plus AIN I	13	7	3	2	2
AIN I only	4	2	—	1	1
HPV infection* plus AIN II	3	—	—	1	2
AIN II only	1	—	—	—	1
Total	112	67	20	12	13

\*HPV infection defined as the presence of koilocytes or a minimum of three of the following features: binucleation, nuclear atypia, orangeophilia, amphophilia, parakeratosis or hyperkeratosis.

only, while 13 had both internal and external anal warts.

Forty two per cent (47/112) showed cytological features indicative of HPV infection (table 1). In 16 cases features of HPV infection and dysplasia (AIN I-II) were present in the same smear, but dysplastic changes only were found in an additional five specimens, bringing the total number of smears with HPV-associated abnormalities to 52 (46%). The most common indicators were bi- or multinucleation and nuclear atypia (found in 36 and 35 smears respectively). Less commonly seen (in order of frequency) were parakeratosis, orangeophilia, hyperkeratosis, amphophilia and koilocytosis. Koilocytes were seen in only 6% (3/47) of the smears displaying at least three of the other cytological features suggestive of HPV infection. A considerable number (13/112) of smears displayed parakeratosis or hyperkeratosis but no other features suggestive of HPV infection: these were placed into a separate category. Overall, almost 20% of the smears showed evidence of mild to moderate dysplasia, with 17 and four of the smears, respectively, displaying features AIN I or AIN II, usually in combination with other cytological features indicative of HPV infection (table 1).

HPV DNA was detected in 40% (45/112) of the anal smear samples by dot hybridisation: HPV 6/11 only was found in 18% (20/112); HPV 16/18 only in 11% (12/112); while HPV 6/11 together with HPV 16/18 was detected in 12% (13/112) of specimens. The relationship between cytological findings and HPV DNA probing is shown in table 1. HPV DNAs (predominantly types 6/11) were found in 13/47 (28%) of the scrapes classified as either normal or inflammatory; in 2/13 (15%) of the scrapes which were para- or hyperkeratotic only; but in 30/52 (58%) of the scrapes with cytological features of HPV infection or AIN. HPV types 16/18 were more commonly detected in scrapes showing dysplastic changes (10/21, 48%) than in those which showed features of only simple HPV infection (10/31, 32%) or showed no specific cytological changes associated with HPV (4/47, 9%).

The overall prevalence of anal HPV infection in this group of men, both clinical and sub-clinical, as indicated by the three different diagnostic techniques—physical examination, cytology and HPV DNA hybridisation—is shown in table 2. If each of the criteria were taken into consideration a total of 73 men (65%) had evidence of clinical or subclinical HPV infection. Comparisons of cytology and DNA hybridisation revealed that 30/52 (58%) cytology-positive scrapes were positive for HPV DNA, whereas 15/60 (25%) cytology-negative scrapes were HPV DNA positive. Of the 29 men with clinical condylomata, cytology failed to detect features of HPV in 11 (40%), while DNA probing was negative

Table 2 Prevalence of clinical and subclinical anal HPV infection in 112 homosexual men as indicated by clinical examination, cytology (HPV infection or AIN, or both) and HPV DNA hybridisation (HPV 6/11 or 16/18, or both)

Clinical status	N	Cytology	HPV DNA	
			N	N
Condylomata present	29	positive	18	positive 12
			11	negative 6
		negative	7	positive 7
			4	negative 4
Condylomata absent	83	positive	34	positive 18
			49	negative 18
		negative	8	positive 8
			39	negative 39

in 10 (34%). However, when the anatomical situation of the warts was taken into consideration a possible explanation for this apparent anomaly became apparent: the 10 men with lesions situated external to the anal canal provided most of both the cytology-negative (7/11) and HPV DNA-negative (6/10) anal canal scrape results. Of the 83 men with no visible warts, 34 (41%) had cytological abnormalities and 26 (31%) were positive for HPV DNA, indicating a high rate of possible subclinical infection.

#### Correlations between HPV infection or AIN and other variables

In order to determine the factors affecting the presentation of anal HPV infection, the distribution of various characteristics was examined among four sub-groups of participants: the 29 men with clinically detectable anal condylomata; the 34 men with cytological or cytological plus molecular evidence suggestive of HPV infection or AIN but without anal condylomata; the 10 men with only HPV DNA detected; and the 39 men with neither clinical, cytological or molecular evidence of HPV infection and/or AIN.

These analyses revealed that the age of the patient was a highly significant factor influencing the clinical presentation of anal HPV infection. Of the 29 patients with clinical anal condylomata, only two (7%) were older than the mean age (34 years). In contrast, older men (aged 35 years or more) were more likely ( $p < 0.01$ ) to present with either no evidence of HPV infection or subclinical infection only (that is, cytological abnormalities and/or a positive result for HPV DNA). There was a negative association between the number of years of homosexual activity and the detection of anal condylomata. None of the patients with condylomata had engaged in homosexual activity for more than 15 years, compared with 17/34 (50%) patients with only cytological/molecular evidence of HPV infection, 10/10 (100%) patients with only HPV DNA detected, and 26/39 (68%) of those who were totally negative by any criteria ( $p < 0.01$ ). Twenty six

(90%) of the patients with current anal warts had a known past history of anal warts, compared with only 15/34 (44%) of those with cytological/molecular features only, 5/10 with HPV DNA only, and 16/39 (41%) of those negative for HPV infection by all three criteria ( $p < 0.01$ ). There was a tendency for men with HIV antibody to present with clinical anal condylomata—16/45 (36%) HIV antibody positive men had warts, compared with 12/67 HIV antibody-negative men (18%)—but these figures just failed to be statistically significant ( $\chi^2 = 3.81$ ,  $p > 0.05$ ). On the other hand, the association between men with clinical syndromes of HIV infection (PGL, ARC and AIDS) and the presence of anal condylomata was highly significant: 9/17 of these men presented with warts compared with 8/95 (8%) men who had no symptoms of HIV infection ( $p < 0.01$ ).

Other variables which were evaluated for an association with anal condylomata were the number of sexual partners over the subjects' lifetime, receptive anal intercourse, past history of penile warts, history of STDs, immune function status, presence of anal dysplasia, and the HPV DNA types detected in the smear. None of these factors were found to be statistically associated with the presence of anal condylomata. All variables were then entered into a logistic regression model. The only factors that were independently associated with the detection of anal condylomata were age less than 34 years (OR 12.9, 95% CI 1.4–16.8); and history of anal warts (OR 11.8, 95% CI 2.8–49.3).

There was no significant association between the presence of clinical warts and AIN, although the proportion of men with anal condylomata who also had dysplastic changes in their anal smears was greater than that of the men with no clinical lesions: 8/29 (28%) versus 13/83 (16%). We were unable to find any statistically significant associations between the presence of AIN and sexual practices, HIV antibody or immune function status, past history of STDs or anal warts or the age of the patient. However, HPV 16/18 was more frequently ( $p < 0.05$ ) found in the scrapes from men with cytological features of AIN I-II: 9/21 (43%) of these scrapes were positive for HPV 16/18, as compared with 3/21 (14%) positive for HPV 6/11 only; and only 16/91 (18%) of the anal scrapes with no cytological evidence of dysplasia were positive for HPV 16/18. Interestingly, in view of the strong association between age and the presence of clinical condylomata, in the group of 21 men with AIN age was not found to be a significant factor: almost equal numbers of men (11 and 10 respectively) were aged either above and below 34 years.

## Discussion

Of this group of 112 homosexual men 73 (65%) had at least one of the diagnostic markers of HPV

infection. The considerable number of cases detected by clinical examination alone (29/112, 26%), emphasises the importance of anoscopy to detect internal anal warts, present in 17% of the men in this survey. The overall high rate of HPV infection was mainly attributable to the addition of anal cytology as one of the diagnostic criteria: an additional 34 cases (41%) of subclinical HPV infection being detected when cytological screening was used as an adjunct to the clinical examination. Other investigators have reported similarly high rates of subclinical anal HPV infection: in their recent survey of homosexual men Haye *et al* found abnormal cytology indicative of HPV infection in 31% (55/178) of men with no macroscopic evidence of anal warts.<sup>5</sup> However, these figures could vary greatly if a different set of cytological criteria were used, as may be the case in other laboratories. Our finding of a very low rate of koilocytosis in anal smears, in contrast to the high rate in cervical smears, indicates that too great an emphasis should not be placed on this feature in smears from the anal canal. In agreement with Morse *et al*, who compared HPV DNA probing with a range of cytological changes in cervical smears,<sup>22</sup> we would conclude that cytological screening for a range of viral associated changes is a more sensitive method of detecting HPV infection than screening for the presence of koilocytes.

After controlling for other variables, we found that visible anal warts were more likely to be detected in young homosexual men, whereas those who were older were more likely to have only cytological or molecular evidence of anal HPV infection (that is, subclinical infection), or totally negative findings. This situation is somewhat analogous to that of skin wart infection, where the highest incidence of infection is in primary school children who are experiencing high levels of primary exposure to cutaneous HPV infection. The fact that certain individuals are susceptible to recurrent or persistent anal HPV infection was highlighted in this survey: a past history of anal warts was a significant factor associated with a current diagnosis of anal HPV infection, using either clinical or cytological criteria, after controlling for other variables. The presence of HIV-associated syndromes (PGL, ARC and AIDS) also correlated with the presence of anal warts, although the presence of HIV antibody per se did not. A recent study by McMillan and Bishop has indicated that persistent anogenital warts are common in HIV antibody-positive homosexual men<sup>23</sup>; while Kiviat *et al*, in a study of 101 bi- or homosexual men with gastrointestinal or anorectal symptoms, found that HPV DNA was more commonly detected in HIV antibody-positive men and in men with a past history of anal warts.<sup>26</sup> Very little is known of the immune responses, either humoral or cell mediated, to HPV infection, but it appears to be type-

specific.<sup>27,28</sup> In the absence of assays to measure immunity to specific types of HPV any hypothesis regarding the nature of effective immunity to these viruses remains impossible to evaluate.

It is now recognised that infection with HPV may be important in the pathogenesis of anal squamous cancers.<sup>1</sup> The frequent association of HPV infection with premalignant changes in the anal canal is indicated by reports such as that of Syrjänen *et al*, who found that histological features of AINI-II were present in 30% of anal condylomas biopsied from homosexual or bisexual men.<sup>8</sup> However, the progression of AIN to invasive cancer has only occasionally been reported, in contrast to the well-documented progression of HPV-associated premalignant lesions (CIN) in the cervix.<sup>29,30</sup> Cytological screening of the anal canal provides a useful technique for early diagnosis of precancerous lesions, but the necessity for routine anal cytological screening remains controversial. In this study, dysplasia was detected in 19% of the smears, with 4% indicating more advanced dysplastic changes (AIN II). In other similar surveys of homosexual men, Frazer *et al* noted cytological evidence of dysplasia in 24 of 61 men (39%) on at least one occasion during a series of visits<sup>4</sup>; whereas Haye *et al* noted "dyskaryosis" in only seven of 221 men (3%).<sup>5</sup> These differences in the rates of dysplasia may be due to either selection bias or to variations in the diagnostic cytological criteria used.

When correlations between various factors and anal dysplasia were sought, only the presence of HPV types 16/18 was found to be statistically significant ( $p < 0.05$ ), with 48% of the dysplastic anal smears positive for HPV 16/18, as compared with only 16% of the non-dysplastic smears. No correlations between dysplastic changes and HIV antibody or immune function (T cell subsets) were present in this group of men, but other workers have reported associations between anal dysplasia and HIV infection.<sup>4</sup> Surprisingly, in view of the latter finding, the incidence of anal dysplasias and cancers does not appear to have risen in proportion to the numbers of homosexual men with HIV infection and immune dysfunction.<sup>25</sup>

The case for routine HPV DNA testing of the anal canal is still in question. DNA hybridisation is an extremely sensitive technique and constitutes an important part of any study of the epidemiology of HPV infection, although little is known of the long-term implications associated with a carrier state of HPV DNA. The results of many published surveys indicate that this technique is more likely than cytology to pick up the cases of latent HPV infection, where cellular morphology may not always be diagnostic.<sup>29,30</sup> A surprising result in the present study was the fact that DNA hybridisation was apparently less sensitive than cytology in detecting HPV infec-

tion in the anal canal: 46% and 40% of smears were shown to be positive by cytology and HPV DNA hybridisation respectively; with the addition of DNA hybridisation testing yielding only an additional 10 positives (9%). This apparent lack of sensitivity may have been partially due to the fact that the anal scrape specimen taken for cytology always preceded that taken for DNA hybridisation, and may have yielded a superior specimen in terms of numbers of epithelial cells. In addition, it is possible that HPV types other than the four used for hybridisation may have been present in some specimens. However, in a comparable study of anal scrape material Kiviati *et al* found HPV DNA in only 17% of their 101 specimens<sup>26</sup>; and Morse *et al*, in their comparison of DNA hybridisation and cytology in cervical scrapes, found detectable HPV DNA in only 39% of scrapes whereas cytological changes suggestive of HPV infection were present in 41%.<sup>22</sup> In summary, it is apparent that no particular diagnostic method is ideal for detecting HPV infection of the anal canal, since a marked lack of correlation was found between clinical criteria, cytology and HPV DNA hybridisation. The percentage of positive cases detected by the different diagnostic methods (using the figure of 73 positive cases as the denominator) was as follows: clinical examination 40%; cytological examination 71%; and DNA hybridisation 62%.

To reduce the transmission of anal HPV infection in the homosexual community, the importance of a thorough clinical examination together with adequate treatment of obvious lesions and follow-up of affected patients cannot be over-emphasised. Unfortunately, the high proportion of men with subclinical HPV infection complicates the situation and it is likely that a significant reduction in the prevalence of this infection will be difficult to achieve. Although cytological screening of the anal canal may provide useful epidemiological information we cannot recommend that this be routinely instituted as a diagnostic tool until the criteria for anal cytology have been standardised and the significance of subclinical anal HPV infection has been determined. The potential of anal dysplasia to progress to invasive anal carcinoma must be defined by prospective studies before any specific recommendations can be made regarding the management of patients with AIN.

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Address for correspondence: Dr C Law, STD Centre, Sydney Hospital, Macquarie Street, Sydney, NSW 2000, Australia.

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