

# Human papillomavirus DNA in the urogenital tracts of men with gonorrhoea, penile warts or genital dermatoses

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## Abstract

**Objective**—To assess the presence of human papillomavirus (HPV) DNA in urethral and urine specimens from men with and without sexually transmitted diseases.

**Design**—Prospective study.

**Setting**—Two London departments of genitourinary medicine

**Patients**—100 men with urethral gonorrhoea, 31 men with penile warts and 37 men with genital dermatoses.

**Methods**—Urethral and urine specimens were taken, HPV DNA extracted and then amplified using the polymerase chain reaction. HPV types 6, 11, 16, 18, 31 and 33 were identified using Southern blotting followed by hybridisation.

**Results**—HPV DNA was detected in 18–31% of urethral swab specimens and in 0–14% of urine specimens. Men with penile warts had HPV detected in urethral swabs more often than did men in the other two clinical groups. “High risk” HPV types were found in 71–83% of swab specimens and in 73–80% of urine specimens containing HPV DNA.

**Conclusions**—HPV is present in the urogenital tracts of men with gonorrhoea, penile warts and with genital dermatoses. In men with urethral gonorrhoea, detection of HPV in urethral specimens is not related to the number of sexual partners, condom usage, racial origin or past history of genital warts. HPV DNA in the urethral swab and urine specimens may represent different aspects of the epidemiology of HPV in the male genital tract. The preponderance of HPV types 16 and 18 in all three groups of men may be relevant to the concept of the “high risk male”.

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## Introduction

Genital warts are the most common of the sexually transmitted viral diseases in the United Kingdom.<sup>1</sup> Evidence suggests<sup>2,3</sup> that the majority of condylomata acuminata are sexually acquired, but the epidemiology of subclinical and latent infections of the genital tract with HPV is less well understood.<sup>4</sup>

DNA from the “genitotropic” HPV types has been isolated from clinically normal cervixes<sup>5</sup> and it has been suggested<sup>6</sup> that carriage

of HPV in adjacent histologically normal tissue may be an important factor in the recurrence of disease after treatment. The male counterpart of carriage of HPV in the absence of disease has not been fully defined, but HPV DNA has been detected in the genital tracts of 5.8% of men with no evidence of condylomata acuminata attending blood donor and dermatology clinics.<sup>7</sup>

Men with urethral gonorrhoea form a well characterised group,<sup>8</sup> whose lifestyles have put them at risk of acquiring sexually transmitted diseases. In an attempt to improve the rational basis for the counselling of such men, we evaluated the frequency of carriage of HPV DNA in their urethras and urine. The results were then compared with those obtained from studying a group of men with penile warts and a group of men with no evidence of sexually transmitted diseases.

## Methods

### *Patients and collection of specimens*

One hundred male patients who attended consecutively one of two genitourinary medicine clinics during the period 1 July 1990 to 31 December 1990 were selected. Selection was on the basis of having symptoms or signs of urethritis, finding intracellular Gram-negative diplococci on the urethral smear, and no condylomata acuminata visible on clinical examination of the genitalia, including the meatus. A urethral sample taken with a 10 µl plastic loop (GIBCO, Paisley, UK) was spread onto a glass slide for Gram staining and subsequently smeared onto a selective medium. Then, the plates were incubated at 37°C in an atmosphere of air and 6% CO<sub>2</sub> before transport to the laboratory for further incubation and identification using standard methods. Men were excluded if *Neisseria gonorrhoeae* was found not to have been cultured from the urethral swab.

After informed consent had been given, a detailed clinical history was obtained using a structured questionnaire. A second urethral sample was taken using a cotton-tipped swab (“STD-Pen for Males”, Abbott Laboratories, Chicago, Illinois, USA) from all of the men. This and the first sample from the two glass urine test were kept for subsequent analysis in 88 of the cases.

Urethral swab and urine samples were also taken in the same manner from 32 men who attended the clinics with penile warts and from 37 men presenting with genital der-

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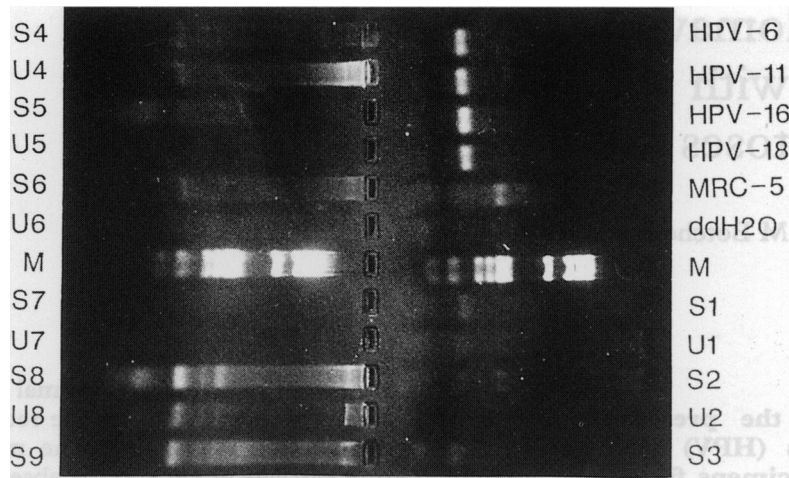


Figure 1 Ethidium bromide staining of agarose gel with positive controls (HPV types 6, 11, 16 and 18), negative controls (MRC-5 and ddH<sub>2</sub>O), molecular weight marker (M) ( $\phi$ X174 DNA cut with *Hae* III enzyme) and specimens of DNA from urine (U) and swabs (S).

matoses but with no evidence of sexually transmitted disease at the time of sampling. None of the men had meatal warts.

#### Processing of specimens

Attempts were made to avoid cross-contamination of DNA products by preparing of reagents, processing patient material, and undertaking the polymerase chain reaction (PCR) and Southern blotting in separate rooms. Positive displacement pipettes or single-use Pasteur pipettes were used to distribute reagents for the PCR and to add the DNA samples to the reaction mixtures. Positive and negative controls were included in each experiment.

#### DNA extraction

Urethral swabs were immersed in 320  $\mu$ l of lysis buffer containing 160  $\mu$ l of 2  $\times$  Summers' buffer / pH 8.0 (40 mM TRIS-HCl, 0.3M NaCl and 20mM EDTA-tetra sodium salt), 100  $\mu$ l double distilled water (ddH<sub>2</sub>O), 32  $\mu$ l pronase E (10 mg/ml), 28  $\mu$ l

of 2.5% sodium dodecyl sulphate (SDS) and 4  $\mu$ l of 0.8 mg/ml tRNA. Urine samples were centrifuged at 3000 rpm for 10 min and the pellet resuspended in 320  $\mu$ l of the same lysis buffer. Samples were incubated at 37°C overnight to allow lysis of the cells and disruption of proteins. DNA was isolated from the specimens by two TE-equilibrated phenol extraction followed by a 24:1 chloroform-isoamyl alcohol extraction and ethanol precipitation for 1 hour at -70°C. The DNA pellet was collected after centrifugation for 10 min at 14 000 rpm, washed briefly with 70% ethanol and dried under vacuum for 30 min. The resulting pellet was then resuspended in 40  $\mu$ l ddH<sub>2</sub>O.

#### The PCR

The method was based on that of Saiki *et al.*,<sup>9</sup> in which general primers GP5 and GP6<sup>10</sup> were used to amplify DNA from the L1 open reading frame of HPV types 6, 11, 16, 18, 31 and 33.

Amplification of DNA was performed using 100  $\mu$ l of a reaction mixture containing 50mM KCl, 10 mM Tris-HCl pH 8.3, 0.01% w/v gelatin, 200  $\mu$ M each of dATP, dCTP, dGTP, and dTTP, 3.5 mM MgCl<sub>2</sub>, 2.5 units of *Taq* polymerase (GIBCO, BRL Life Technologies, Uxbridge, Middlesex, UK) and 50nM each of GP5 and GP6. The mixture was overlaid with several drops of mineral oil to prevent evaporation and subjected to 40 cycles of amplification using a PCR processor (Hybaid, Teddington, UK). Each cycle included denaturation at 94°C for 20 seconds, primer annealing at 40°C for 60 seconds and chain elongation at 72°C for 20 seconds.<sup>11 12</sup> Samples containing 100 pg of the full length genome of HPV types 6, 11, 16 and 18 were used as positive controls. Negative controls were a fibroblast line (MRC-5) and ddH<sub>2</sub>O (fig 1). Serial dilutions of HPV DNA were tested and the detection limit was found consistently to be 10<sup>-14</sup>g or less of DNA. This corresponded to approximately 10 molecules of HPV DNA/100  $\mu$ l of reaction mixture.

#### Southern blotting

20  $\mu$ l of each of the products of the PCR were analysed by using agarose gel electrophoresis with ethidium bromide staining.  $\phi$ X174 DNA cut with *Hae* III restriction endonuclease was used to enable estimation of the sizes of the PCR products. Southern blotting was performed using a modification of standard protocols.<sup>13</sup> The agarose gel was denatured for 30 min in 1.5M NaCl/ 0.5M NaOH, followed by neutralisation for 60 min in 1.5M NaCl/0.5M Tris pH 7.5/1mM EDTA. The DNA products were then transferred from the agarose gel to nylon membranes (Gene-Screen Plus, DuPont, USA) by a capillary blot procedure using 6  $\times$  SSC (0.9M NaCl, 0.09M sodium citrate) as the transfer buffer.

Hybridisation for the detection of HPV types 6, 11, 16, 18, 31 and 33 was performed at 55°C for 16 to 24 hours using oligonucleotide probes which had been end-labelled

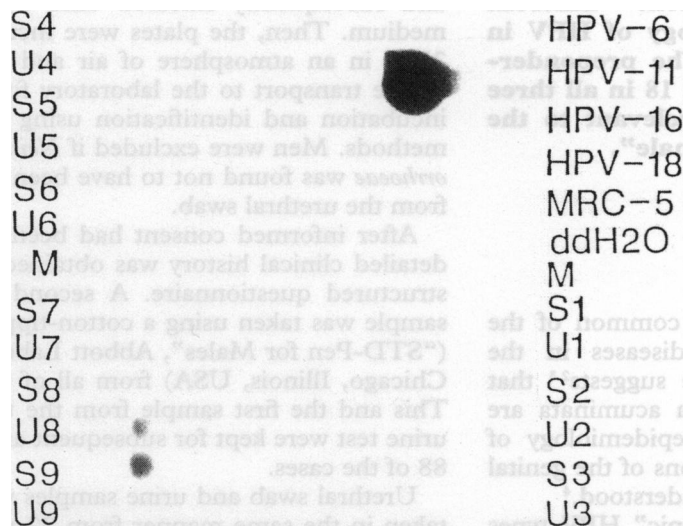


Figure 2 Hybridisation of PCR products with HPV type 11-specific probe on nylon membrane following Southern blot. Abbreviations as for fig 1. Positive result seen for HPV type 11 control, urine no. 8 and swab no. 9.

Table 1 Types of HPV DNA found in urethra and urine of men with urethral gonorrhoea

HPV type	No of specimens positive for indicated type		
	Swab (n = 18)	Urine (n = 11)	Swab or Urine (n = 25)
6	5 (28)*	3 (27)	7 (28)
11	8 (44)	6 (54)	13 (52)
16	6 (33)	6 (54)	10 (40)
18	7 (39)	2 (18)	8 (32)
31	2 (11)	0	2 (8)
33	2 (11)	0	2 (8)

\*Figures in brackets are percentage of swab or urine specimens with HPV DNA present.

with  $^{32}\text{P}$ -labelled dATP. Hybridisation was undertaken in a solution containing  $6 \times \text{SSC}$ ,  $20 \times \text{Denhardt's}$  solution (0.4% bovine serum albumin, 0.4% polyvinyl pyrrolidone, 0.4% Ficoll 400), 0.5% SDS, 1mM EDTA-disodium salt and 50  $\mu\text{g/ml}$  salmon sperm DNA (Sigma, Dorset, U.K.) To remove non-specifically bound probe, the Southern blots were washed in low stringency conditions of  $2 \times \text{SSC}/0.1\%$  SDS for 5 min at room temperature, followed by one wash with  $2 \times \text{SSC}/0.1\%$  SDS for 5 min at  $55^\circ\text{C}$ . Autoradiography was performed for 24 hours on Kodak X-OMAT S film between intensifying screens at  $-70^\circ\text{C}$ . An example of this is shown in fig 2.

The oligonucleotide probes (prepared using a Milligen/Biosearch 7500 DNA Synthesizer) were:

HPV 6 5' ACA TGC GTC ATG TGG AAG AGT 3'

HPV 11 5' TAA ATC TGC TAC ATA CAC TAA 3'

HPV 16 5' TCT ACT TCA GAA ACT ACA TAT 3'

HPV 18 5' TGC TTC TAC ACA GTC TCC TGT 3'

HPV 31 5' CAC ACA AGT AAC TAG TGA CAG 3'

HPV 33 5' TCT GTT TGT GCT GCA ATT GCA 3'

## Results

*Demographic and clinical features of the patients*  
Seventy-six of the 100 men with urethral gonorrhoea described themselves as heterosexual, 19 homosexual, and five bisexual. Their age range was 17.0 to 55.6 years (mean 26.7 years). Fifty-two of the men were Caribbean, 34 Caucasian, eight African and four from

other racial groups. Information on the ethnic origin of two men was not available. The mean number of lifetime sexual partners was 43, with a range of 3 to 200; in addition, one bisexual man claimed to have had 1000 lifetime sexual partners. A history of genital warts was given by six of the men. One of 85 of the men who gave blood specimens at the time of presentation had serological evidence of syphilis. Fifty-eight of the men were cigarette smokers. Of the 98 men who provided information on condom usage, 27 claimed always, 59 sometimes and 12 never to have used them.

The demographic and clinical features of the men with penile warts or with no evidence of sexually transmitted diseases were not appreciably different from the men with gonorrhoea.

### Types of HPV detected in swab and urine specimens from men with gonorrhoea

Eighteen of 100 urethral swabs and 11 (12.5%) of 88 urine specimens that were obtained contained HPV DNA. Overall, HPV DNA was detected in one or other specimen from 25 men; this comprised 15 (25.8%) of the 58 cigarette smokers and 10 (23.8%) of the 42 non-smokers. HPV types 11, 16 and 18 were the most common in both types of specimen (Table 1). The different combinations of types of HPV found in the specimens is shown in Table 2. DNA from HPV types 16, 18 31 or 33 (the so-called "high risk" types) was found in 15 (83%) of 18 swabs and in 8 (73%) of 11 urine specimens in which any HPV DNA could be detected.

### HPV in relation to demographic and clinical details of men with gonorrhoea

The occurrence of HPV types in urethral or urine specimens was not associated significantly with racial origin, the number of lifetime sexual partners, a history of non-genital warts, condom usage, or whether cigarettes were smoked.

Twenty-one (28%) of the 75 men who were 30 years or less had detectable HPV, as compared to four (16%) of the 25 men who were older. Twenty-two (29%) of the 76 heterosexual men, three (16%) of the 19 homosexual men and none of the five bisexual men had HPV detectable. One (17%) of the six men with a history of genital warts had HPV present.

Table 2 Single and multiple types of HPV found in urethra and urine of men with gonorrhoea

HPV type	No of specimens positive for indicated type	
	Swab (n = 18)	Urine (n = 11)
6 alone	0	0
11 alone	3	3
16 alone	3	3
18 alone	4	2
31 alone	0	0
33 alone	0	0
6 and 11	3	0
6, 11 and 16	1	3
6 and 18	1	0
11, 16 and 18	1	0
16, 18, 31 and 33	1	0
31, 33	1	0

Table 3 Detection of HPV in urethral swab and urine specimens in relation to clinical group

Specimen type	Proportion (%) of specimens positive in patients with		
	Urethral gonorrhoea	Penile warts	Genital Dermatoses
Urethral swab	18/100 (18)	10/32 (31)	7/37 (19)
Urine	11/88 (13)	0/5	5/37 (14)
Urethral swab or urine	25/100 (25)	10/32 (31)	9/37 (24)
<i>"High risk" HPV types</i>			
Urethral swab specimen	15/18 (83)	8/10 (80)	5/7 (71)
Urine specimens	8/11 (73)	0/0	4/5 (80)

#### *Detection of HPV in relation to clinical group*

The results of testing urethral and urine specimens from men with gonorrhoea are compared with those from men with penile warts and with genital dermatoses in Table 3. The occurrence of HPV in the specimens from the men and the proportion of those with "high risk" types appear to be independent of clinical status.

#### **Discussion**

The results of several studies<sup>14-18</sup> have shown that 20%<sup>16</sup> to 32%<sup>14</sup> of men presenting to genitourinary clinics with genital warts have evidence of at least one other sexually transmissible disease (STD). In particular, the incidence of gonorrhoea in such men varies from 0%<sup>17</sup> to 9.9%.<sup>14</sup> These studies rely on the clinical identification of condylomata acuminata, which probably represent only a minority of HPV infections of the anogenital area<sup>19</sup> and they do not address the subject of this communication, namely the question of how often men with gonorrhoea are infected by HPV.

The use of DNA technology, in particular the PCR, has led to the detection of HPV in a large proportion of cervical scrapes from women with and without clinically apparent disease.<sup>5</sup> Understanding the epidemiology of HPV infection of the male genital tract has, however, been hampered by the lack of a satisfactory counterpart to the cervical scrape. Urethral cytology<sup>20-22</sup> and HPV antigen detection<sup>20 22 23</sup> have been found insensitive for detecting HPV. Furthermore, there seems to be a poor correlation in men between cytological abnormalities and the presence of HPV DNA.<sup>24</sup> Thus, the question of what constitutes an appropriate approach to sampling in men is pertinent.

Anogenital infection with HPV seems to be multifocal<sup>25</sup> and there is evidence of widespread subclinical infection which may be visualised by acetic acid staining.<sup>26</sup> However, this method is neither specific<sup>27 28</sup> nor sensitive,<sup>29</sup> and its general introduction for the screening of men would have significant resource implications with no clearly established benefit.<sup>30 31</sup>

In contrast, samples obtained by applying abrasive pads and brushes to the male genitalia<sup>19 32</sup> have enabled specific detection of HPV. The majority of urethral condylomata acuminata develop in the distal 1 cm of the urethra<sup>33</sup> and it has been postulated<sup>32</sup> that this area of the urethra may be analogous to the transitional zone of the cervical epithelium and might act as a reservoir for infection with HPV.<sup>22</sup> In view of these observations, the finding<sup>19</sup> that urethral swabs are as effective as abrasive pads in detecting infection with HPV in male genitalia, and that the men had a urethral discharge, we undertook urethral sampling of our patients. Cotton wool-tipped swabs, designed originally for the detection of *C. trachomatis*, were used, as they have been found previously to be acceptable to patients and effective in sampling the urethral epitheli-

um for urogenital pathogens. As several groups<sup>11 34 35</sup> have detected HPV DNA in urine, examination of urine also formed part of our study.

The PCR, when used under carefully controlled conditions, is currently the most sensitive technique for the detection of HPV,<sup>36</sup> allowing detection of very few viral particles. Sample size, therefore, may be small and genital trauma minimised. As great care was taken during the collection of clinical material, and both positive and negative controls were used at all stages of analysis of the specimens, we are confident that the results reflect the true occurrence of HPV in the specimens, rather than contamination. Our detection of HPV DNA in the urogenital tracts of 24-31% of men attending the clinics compares with the finding of HPV DNA in the genital tracts of 31 (5.8%) of 530 men attending a blood donation centre and a dermatology department,<sup>7</sup> and of 7 (8%) of 89 Swedish army recruits,<sup>29</sup> none of whom had clinical evidence of anogenital warts. Our findings are consistent with the 20% of men attending STD clinics in the UK<sup>24</sup> and the USA<sup>37</sup> without evidence of condylomata acuminata but with HPV DNA detectable in genital swabs. One other group of workers<sup>19</sup> found HPV DNA in 191 (84%) of 228 men attending a Swedish STD clinic. The validity of this result has yet to be confirmed, the authors accepting that they may have overestimated the incidence.

In an attempt to assess the significance of finding HPV in a proportion of the urethral swab specimens from men with gonorrhoea, we investigated two other groups of men from the same clinics. Men with penile warts were included in view of their clinically apparent infection with HPV and because of evidence<sup>38</sup> suggesting that HPV may be found contaminating the urethra as a result of passive transfer from adjacent warts. We also included a group of men with no sexually transmitted disease at the time of sampling, that is, those with genital dermatoses. Of particular note is the observation that similar proportions of men with gonorrhoea and men with no sexually transmitted disease had HPV DNA detected in both their urethral swab and urine specimens. Furthermore, this observation suggests that urethral irritation per se in men with gonorrhoea is not a significant factor in the shedding of HPV from the urethra. The small number of urine specimens taken from men with penile warts makes interpretation of the results for this group of men difficult.

The detection of HPV DNA in the urine of 0-14% of the men in our study contrasts with that in the urine of 15 (88%) of 17 men with meatal warts and none of 14 healthy controls in one study,<sup>11</sup> and with that in the urine of two (22%) of 9 men whose partners had HPV infection.<sup>34</sup> Condylomata acuminata occur rarely in the bladder<sup>39</sup> and there have been conflicting reports of HPV DNA in the prostate.<sup>40 41</sup> As only a small proportion of men in each clinical group had HPV DNA

detectable in both the urine and urethral swab specimens, it may be that these different sampling techniques enable results in assessment of different aspects of infection of the urogenital tract with HPV.

The significance of detecting HPVs in the male urogenital tract is difficult to determine. It is possible that a few viral particles might remain on the genitalia from recent sexual contact, and even from underwear,<sup>42</sup> without actually representing active infection. The natural history of subclinical HPV infection and, in particular the infectiousness for sexual partners, is also unknown. Indeed, it is currently unclear whether subclinical HPV infection is sexually acquired<sup>43</sup> as DNA of the genitotropic HPV types has been found in virgins,<sup>43</sup> the foreskin of neonates<sup>44</sup> and on fomites.<sup>45</sup> The lack of correlation between the presence of HPV DNA and the number of life-time sexual partners of the men seen in our study argues against sexual transmission, as does the reported condom usage, although the reliability of such reported behaviour is clearly open to question. Furthermore, the minor differences that we detected in the rates of HPV infection in men with and without sexually transmitted diseases suggest that sexual lifestyle may not be a major factor in determining infection with HPV. However, the greater incidence of HPV in men in younger age groups is consistent with sexual transmission. Other authors<sup>24-37</sup> have also found ambiguous evidence of sexual transmission in clinically inapparent infections.

Our finding of a larger proportion of "high risk" HPV types than "low risk" types in asymptomatic infections normally found in condylomata acuminata, is consistent with the results of other studies.<sup>24-26, 37</sup> Furthermore, our observations were independent of both the sample type and patient group, and may be relevant to the concept of the "high risk male", whose sexual partner(s) are at increased risk of cervical dysplasia.<sup>46-47</sup> In view of the evidence linking infection with HPV to anogenital neoplasia in both women and men,<sup>48</sup> further work needs to be done to fully evaluate the various methods for detecting HPV infection. In particular, the role of sexual activity in the acquisition and maintenance of genital HPV infection needs to be clarified. Only then will it be possible to understand the nature of this condition, and to develop rational management strategies.

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