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## Lesional HPV types of cutaneous warts can be reliably identified by surface swabs

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### ABSTRACT

**Background:** Large numbers of HPV types infect the human skin and members from the HPV genera alpha, gamma and mu are associated with cutaneous warts.

**Objectives:** The aim of this study was to test if the HPV genotypes in swabs of the overlying skin are identical to the types present within these warts.

**Study design:** To this purpose, 25 persons being treated for persistent cutaneous warts were enrolled. Swabs of the overlying skin of the wart were collected from each participant. Additionally, scabs of the wart and deeper portions of the warts were surgically removed. HPV genotyping was performed on all samples using the novel HSL-PCR/MPG assay and the HPV genotyping results were compared.

**Results:** From the 25 wart biopsies one was HPV negative. 15 were positive for HPV27, 3 for HPV57, 2 for HPV2, 2 for HPV1, 1 for HPV3 and 1 wart biopsy was positive for both HPV41 and HPV65. Scabs and swabs of the warts both showed identical typing results as the biopsies in 24 of the 25 cases (sensitivity: 96%).

**Conclusions:** There was an excellent agreement between HPV types in the swabs of the skin that overlies the warts and the biopsies of these warts validating the use of wart swabs for future studies of wart-associated HPV types. HPV27 was highly prevalent (70%) in the in adults of the investigated population of patients with persistent cutaneous warts.

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## 1. Background

Large numbers of HPV types, distributed over five papillomavirus genera, infect the human skin.<sup>1</sup> HPV types belonging to four of those genera, i.e., *Alphapapillomavirus*, *Gammapapillomavirus*, *Mupapillomavirus* and *Nupapillomavirus* have been associated with cutaneous warts, mainly with foot and hand warts.<sup>2–13</sup> Reliable detection and sampling techniques are mandatory to be able to study the epidemiology of these HPV infections.

The hyperkeratotic skin lesion (HSL) PCR/MPG assay has been described recently<sup>14</sup> and it detects and identifies DNA of all known wart-associated HPV types from the alpha-(HPV2, 3, 7, 10, 27, 28, 29, 40, 43, 57, 77, 91 and 94), gamma-(4, 65, 95, 48, 50, 60 and 88), mu-(HPV1 and 63) and nu-genus (HPV41). Biopsies of the lesions are the gold standard for determining which HPV type is present within the warts. However, taking a biopsy and extracting the DNA

is not only labour intensive and painful for the patient, but will also be subject of ethical restraints because of the benign nature of these warts. Large-scale epidemiological studies, therefore, should not rely on biopsies only. If non-invasive samples, like wart swabs were a good target for detection of HPV types causing the specific wart, larger studies could be more easily done to investigate the epidemiology of these HPV types. In previous work,<sup>15–17</sup> focused on healthy skin, actinic keratoses, basal cell and squamous cell carcinomas it was shown that skin swabs are an efficient target for the detection of cutaneous HPV types from the beta and gamma genera, but, so far cutaneous warts and their associated HPV types were not evaluated.

## 2. Objectives

The aim of this study was to validate the use of swabs of the skin that overlies the wart for reliable detection of the HPV type(s) present in the deeper portions of the same wart. Our expectation was that these swabs would contain the same HPV type(s) as the underlying warts. Swabs from the perilesional skin and from the forehead were taken to investigate whether the HPV

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DNA found in the wart was also detectable elsewhere on the body.

### 3. Study design

#### 3.1. Clinical materials

Twenty-five consecutive immunocompetent persons seeking treatment for persistent cutaneous warts in general practice (8) or outpatient dermatology department (17) were enrolled.

Swabs of the overlaying skin of the wart were taken from each participant by firmly rubbing a pre-wetted cotton-tipped stick for 5 times over the surface of the lesion (Swab of wart, Table 1). Similarly, swabs of the skin area around the wart (Swab perilesional, Table 1) and the forehead (Swab forehead, Table 1) were collected by sampling a skin area of 5 by 5 cm. Next, the cotton-tipped sticks were put in 1 ml of saline solution, moved around in the solution and were pressed against the inner part of the tube to remove most of the liquid in the cotton-tips. Ten  $\mu$ l of the saline solution was used directly in the novel HSL-PCR/MPG assay. In order to reduce the likelihood of cross contamination during sample taking first the swabs of the forehead, then the perilesional swabs and finally the swabs of the warts were taken. Additionally, scabs of the wart and deeper portions of the warts were surgically removed and incubated in 100–250  $\mu$ l of a 1 mg/ml proteinase K solution at 70 °C for 16 h to release the DNA, after which, proteinase K was inactivated at 95 °C for 10 min. All samples were analysed in random order.

#### 3.2. HSL-PCR/MPG assay

The HSL-PCR/MPG assay was performed as described by the manufacturer (Labo Bio-medical Products BV, Rijswijk, the Netherlands). Briefly, it comprises a primer set with 27 non-degenerate primers (13 forward and 14 reverse) generating a biotinylated amplicon of 76–84 bp from the L1 region in 35 ampli-

fication cycles. Genotyping is performed with bead-based xMAP suspension array technology which is able to simultaneously identify 23 wart-associated HPV types from the alpha-(HPV2, 3, 7, 10, 27, 28, 29, 40, 43, 57, 77, 91 and 94), gamma-(4, 65, 95, 48, 50, 60 and 88), mu-(HPV1 and 63) and nu-genus (HPV41).<sup>14</sup> The assay design results in very low aspecific background signals and therefore requires no background subtraction. Cut-off's were applied as described before.<sup>14</sup> Negative extraction, PCR and genotyping controls were incorporated and remained negative upon analysis with the HSL-PCR/MPG assay.

#### 3.3. Statistical analysis

The sensitivity of HPV genotyping on the different swabs and the wart scabs was determined by comparison with the HPV genotyping results generated from the wart biopsies. We did not test the specificity of the swabs, because we did not intentionally include skin lesions that were not clinically diagnosed as cutaneous warts.

### 4. Results

The study population was composed of 25 participants, including 11 women and 14 men. Their mean age was 31.7 years, ranging from 6 to 53 years (Table 1). Seven of them had hand warts and the other 18 had foot warts.

Twenty-four of the 25 wart biopsies (96%) were HPV positive (Table 1). 15 were positive for HPV27, 3 for HPV57, 2 for HPV2, 2 for HPV1 and 1 for HPV3. One wart biopsy was positive for both HPV41 and HPV65 and one was HPV negative. Remarkably, in the adult participants 70% (14 of 20) of the HPV positive warts contained HPV27 (Table 1).

Genotyping results obtained from scabs and swabs of the warts were identical to the results from the deeper wart portions in 24 of the 25 (96%) cases (Table 1). In two cases the result between the wart biopsy and scab of the wart or wart swab were not identical (Table 1). The scab and wart swab were negative in these two cases

**Table 1**

HPV genotyping results obtained with the HSL-PCR/MPG assay. Results indicated in bold font are not identical to the result from the wart biopsy.

Participant	Age in years	Location wart	Wart biopsy	Scab	Swab of wart	Swab perilesional	Swab forehead
1	25	Hand	27	27	27	27	27
2	19	Foot	27	27	<b>Negative<sup>a</sup></b>	<b>2, 27</b>	<b>2, 27, 57</b>
3	27	Foot	27	27	27	27	<b>3</b>
4	19	Hand	3	3	3	3	<b>Negative</b>
5	62	Hand	Negative	Negative	Negative	<b>48</b>	<b>48</b>
6	23	Foot	27	27	27	27	<b>2, 27</b>
7	41	Foot	2	2	2	2	2
8	42	Foot	27	27	27	27	27
9	25	Hand	27	27	27	27	27
10	21	Hand	27	27	27	27	27
11	46	Foot	2	2	2	2	2
12	44	Foot	57	<b>Negative<sup>a</sup></b>	57	57	<b>Negative</b>
13	53	Foot	27	27	27	27	27
14	48	Foot	27	27	27	27	<b>Negative</b>
15	42	Foot	27	27	27	27	<b>Negative</b>
16	34	Foot	27	27	27	27	<b>Negative</b>
17	30	Hand	57	57	57	57	<b>Negative</b>
18	49	Foot	57	57	57	57	<b>Negative</b>
19	19	Foot	27	27	27	27	27
20	53	Foot	27	27	27	27	<b>Negative</b>
21	35	Hand	27	27	27	<b>Negative</b>	<b>Negative</b>
22	11	Foot	41, 65	41, 65	41, 65	<b>1, 41, 65</b>	<b>10</b>
23	6	Foot	1	1	1	1	1
24	7	Foot	1	1	1	<b>1, 2</b>	<b>Negative</b>
25	10	Foot	27	27	27	<b>Negative</b>	<b>Negative</b>

<sup>a</sup> These two samples show an elevated signal for HPV27 (119 MFI) and HPV57 (134MFI), respectively that is well above the background signal of the probe but still below the cut-off of the assay for positivity (200MFI).

but clearly showed an elevated signal for the same HPV type present in the wart biopsies. This results in an equal sensitivity of 96% for the scabs and swabs of the warts.

Swabs taken from around the wart and from the forehead provided identical HPV genotyping results as the wart biopsy for 19 (19/25, 76%) and 9 (9/25, 36%) participants, respectively. In the perilesional and forehead swabs 3 and 2 multiple infections were detected and 2/25 and 11/25 were HPV negative, respectively (Table 1). The resulting sensitivity of perilesional swabs was higher than the sensitivity of forehead swabs at 92% and 46%, respectively.

One of the warts (participant 5) was HPV negative with the HSL-PCR/MPG assay. Additional PCR and direct sequencing analyses revealed the presence of HPV107, a *Betapapillomavirus* (BetaPV) type, in the swab of the forehead, perilesional, in the swab and the scab of the wart, but not in the deeper portion of the wart. BetaPV are ubiquitous viruses<sup>16,18–20</sup> and the clinical relevance of finding HPV107 in this participant with respect to the wart was further investigated. In retrospect, the wart-like lesion consisted of an accumulation of callus as a result of bagpipe playing, and did not have a viral genesis.

Limited data are available about methodologies of preparing skin wart samples for PCR analyses. Therefore, we used samples from the first ten participants for limited optimization experiments. Pre-treatment of wart swabs by proteinase K digestion or manual column based DNA purification did not result in additional positivity as compared to direct use of 10 µl of swab sample in the PCR (data not shown). For the scabs and deeper wart portions the proteinase K treatment as described above was optimal as compared to washing the material in 200 µl of water and using 10 µl of water either directly in the PCR or after an incubation of 5 min at 100 °C (data not shown).

## 5. Conclusions

In 24 of the 25 cases (96%), swabs of the overlying skin showed the same HPV genotyping result as the underlying wart. The use of these swabs is therefore a highly sensitive sampling technique for the determination of the HPV type in cutaneous warts and this method could be reliably and easily used in large scale epidemiological studies. Scabs could also be used because this method is equally reliable, but this method is more invasive and more prone to contamination and more difficult to standardize.

In the two cases that the genotyping result of the wart biopsy was not confirmed by the genotyping result of the scab of the wart or of the wart swab (participant 2 and 12, Table 1) there was a clearly elevated signal visible. These two results illustrate that cut-offs are difficult to establish for assays based on xMAP suspension array technology. Apparently, there is a 'grey-zone' for each probe signal and the use of a cut-off will lead to an underestimation of positivity.

Genotyping performed on swabs from perilesional skin is less sensitive and using swabs from the forehead is much less sensitive, so these locations should not be used for the determination of HPV type in the wart. In addition, the agreements in genotyping results between perilesional skin swabs and forehead swabs and wart biopsies were lower at 76% and 36%, respectively.

Even though the agreement between the genotyping results of the wart biopsies and swabs of the overlying skin was very high in this study, the swabs need to be taken very carefully if warts exist in close proximity of each other as the samples might get cross-contaminated if these warts harbor different HPV types.

Remarkably, in the adult participants 70% (14 of 20) of the HPV positive warts contained HPV27. It should be noted that the participants of this study, enrolled in an outpatient dermatology department and in general practice were treated for persisting warts whereas skin warts usually resolve spontaneously. It could therefore be speculated that HPV27 is especially associated with persisting skin warts in immunocompetent adults.

In conclusion, the data presented in this study show that HPV genotyping in swabs taken from the surface of persistent cutaneous warts accurately identifies the HPV type that is present in the biopsy from the deeper wart portions and thus provide a useful research tool for epidemiological studies.

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None.

## Conflicts of interest

The authors declared no conflicts of interest.

## Ethical approval

None required because procedure was part of clinical patient care.

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