Acta Medica Okayama

Volume 41, *Issue* 5 1987 *Article* 8

OCTOBER 1987

A Rapid Method for the Detection of Papillomavirus in Warts : The Fre-quency of Virus Detection in Various Types of Warts

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Abstract

A rapid method was devised for the detection of virus particles in wart specimens. The upper layer of warts was cut perpendicularly to the surface, and the freshly cut surface was lightly touched to an electron microscope grid. The grid was then stained with a small drop of phosphotungstate and observed electron microscopically. On the specimen grid thus prepared, papillomavirus particles were easily discriminated from tissue debris. Papillomavirus particles were detected in 71% of verrucae plantares, 78% of verrucae palmares, 50% of verrucae vulgares and 75% of condylomata acuminata by the present method.

KEYWORDS: papillomavirus, warts, electron microscopy

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*PMID: 2825468 [PubMed - indexed for MEDLINE]

Acta Med Okayama 41 (5) 233-235 (1987)

— Brief Note —

A Rapid Method for the Detection of Papillomavirus in Warts: The Frequency of Virus Detection in Various Types of Warts

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A rapid method was devised for the detection of virus particles in wart specimens. The upper layer of warts was cut perpendicularly to the surface, and the freshly cut surface was lightly touched to an electron microscope grid. The grid was then stained with a small drop of phosphotungstate and observed electron microscopically. On the specimen grid thus prepared, papillomavirus particles were easily discriminated from tissue debris. Papillomavirus particles were detected in 71% of verrucae plantares, 78% of verrucae palmares, 50% of verrucae vulgares and 75% of condylomata acuminata by the present method.

Key words : papillomavirus, warts, electron microscopy

To study the etiology of papilloma or papilloma-like lesions, it is essential to detect papillomavirus in the tissue. Due to the lack of a propagation system for this virus, the detection of human papillomavirus (HPV) has long been done by electron microscopy (1-4). Although immunological or biochemical methods have also been applied recently (4-8), electron microscopy is still a very reliable method for the detection of HPV. The electron microscopic detection of HPV has usually been done with ultrathin sections of resin-embedded tissues or with tissue extracts purified by ultracentrifugation (1, 2, 4). The preparation of ultrathin sections or purified tissue extracts involves complicated and time-consuming procedures, and is not suited to the rapid examination of many specimens obtained clinically. To our knowledge, the method of Chambers *et al.*, in which the tissue is soaked and teased apart in phosphotungstate (PTA) solution in a watch glass, is the simplest one reported so far (3). We devised a simpler and quicker method, and applied it to the study of the frequency of detection of virus particles in banal types of warts.

The upper layers of warts removed prior to the freezing treatment were preserved at −80°C until examined. Electron microscope grids were coated with Formvar and carbon and cleaned in an Ion Cleaner, Eiko Engineering Co., Ibaragi, Japan, to make the surface hydrophilic. Each specimen was thawed and cut with a razor blade or scissors perpendicularly to the surface. The central area of the freshly cut surface was lightly touched to a grid. A small drop of 1% PTA (pH 6.8) was put on the grid, left for about 10 sec and blotted, and the grid was inserted in the electron microscope. Electron microscopic observation was done as described previously (9, 10). A Hitachi HS-9 electron microscope was used at the

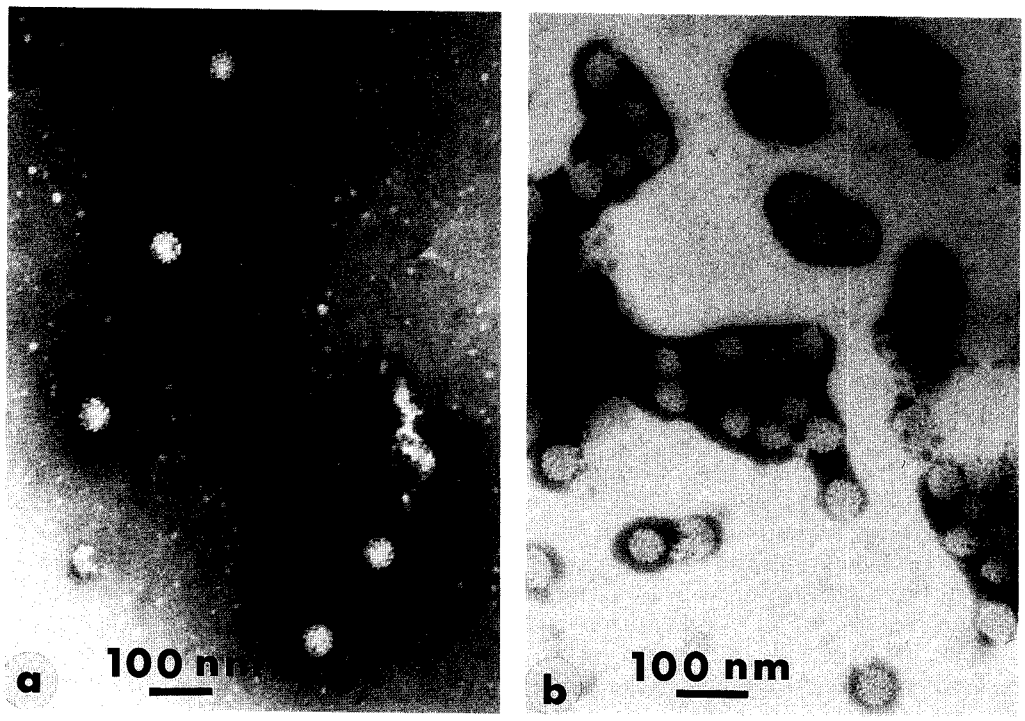

Fig. 1 Negatively stained human papillomavirus particles in the specimens prepared by the rapid method. a: Observed immediately after preparation. b: Prepared and preserved in a desiccator at room temperature for a week and observed; the images of virus particles are deteriorated, but still clearly discernible.

magnifications of 20,000-50,000, operating at 75 kV.

On the specimen grid thus prepared, papillomavirus particles were easily discriminated from tissue debris by their uniform spherical shape, uniform size of about 55 nm and characteristic surface structure studded with capsomeres (Fig. 1a). When the specimen grids were not observed immediately after preparation and were preserved in a desiccator at room temperature for a week to 3 months, the images of virus particles deteriorated somewhat. However, virus particles were still clearly discriminated from tissue debris (Fig. 1b).

Table 1 The types of warts and the frequency of detection of virus particles[a]

Types of warts	No. of specimens examined[b]	No. of specimens showing virus particles (%)
Verruca plantaris	42	30 (71)
Verruca palmaris	9	7 (78)
Verruca vulgaris	107	53 (50)
Condyloma acuminatum	4	3 (75)

a: The presence of virus particles was examined by the rapid method.
b: All specimens were from different patients. For each specimen, one grid was prepared and examined.

The presence of virus particles in various types of human warts was examined by the method described above. When specimens were obtained from a patient with multiple warts, only one of warts was examined as a representative. As shown in Table 1, virus particles were detected in 30 (71%) of 42 verrucae plantares, 7 (78%) of 9 verrucae palmares, 53 (50%) of 107 verrucae vulgares and 3 (75%) of 4 condylomata acuminata.

To compare the present rapid method and

purification methods with regard to reliability, tissue extracts were prepared from 5 specimens which were determined to be virus-free by the present method, and purified by differential centrifugations as described previously (4). The purified tissue extracts were stained with 1% PTA and examined electron microscopically. Virus particles were again not detected in all 5 specimens, suggesting that the purification methods are not necessarily more reliable than the present method. It is conceivable that a considerable portion of the virus particles are lost by drastic purification procedures, making their detection, particularly in small specimens, not so efficient. Barrera-Oro and co-workers extracted and collected virus particles from warts by sophisticated extraction procedures and ultracentrifugation, and detected virus particles in 22 (55%) of 40 warts, of which the types were not described (11). On the other hand, Chambers and co-workers detected virus particles in 6 of 6 human cutaneous warts by their simple teasing method (3).

As described above, our present method is very simple and quick, and yielded fairly good results in the detection of virus particles in wart specimens. It thus appears that the present method is applicable to the rapid preliminary examination or screening of clinically obtained specimens for HPV particles. Another advantage of the present method is that no tissues are lost by the examination itself, thus leaving all the specimens usable for other experiments such as virus purification.

Acknowledgments. The authors are grateful to Professor Nohara and associates of the Department of Dermatology, Professor Ohmori and associates of the Department of Urology, and Professor Sekiba and associates of the Department of Obstetrics and Gynecology, Okayama University Medical School for supplying specimens. The publication cost of this paper was defrayed by a grant from our previous colleagues who had worked in our laboratory.

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Received: June 10, 1987
Accepted: July 14, 1987

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