doi:10.1017/S1431927611002315
Microsc. Microanal. 17 (Suppl 2), 2011

Quality Assurance Testing of Human Papilloma Virus-Like Particles by Negative Stain Electron Microscopy

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Cervical cancer is highly curable when detected early. However, it remains one of the leading causes of cancer related death in women worldwide [1, 2]. The etiological link between human papillomaviruses (HPV) and cervical cancer has led to improved cervical cancer screening and to development of vaccines against HPV infection. Highly effective recombinant virus-like particle (VLP) based subunit vaccines have been released that target either HPV16, 18, 6, and 11(*Gardasil*™) or HPV16 and 18 (*Cervarix*™) [3]. HPV16 and 18 are associated with the majority of cervical intraepithelial neoplasias (CIN). HPV6 and 11 are less associated with CIN but highly associated with anogenital warts.

Unlike similarly structured polyomaviruses, HPV cannot be grown in conventional cell culture. This problem complicates specific antigen production for vaccine development, and immunodiagnostic studies. Fortunately, the genomes of the major pathogenic HPV types are well characterized. The availability of these sequences facilitates recombinant protein expression methods to produce specific capsid proteins (L1 alone or L1 and L2) that self-assemble to form VLPs which are structurally similar to HPV (FIGS. 1, 2) [3]. The morphology of HPV VLPs does not vary significantly by types. We have seen more of the tubular forms associated with HPV 16 VLPs (FIG. 2) than with HPV type 6, 11, and 18 VLPs (FIG. 1), although limited sampling may not allow this observation to be significant. The VLPs can be produced in insect cells, yeast, bacteria, or human cells. Once the VLPs are expressed they may be purified by traditional protein purification techniques including gel filtration, gradient centrifugation in sucrose, cesium chloride, or iodixanol (Optiprep™). They are then used for developing vaccines, or immunodiagnostic tests. HPV VLPs have been well characterized by negative stain transmission electron microscopy (EM) as well as by their physico-chemical, immunologic, and stability properties [4]. Prior to their use for test development, we evaluate the VLPs for quality and quantity by using EM. EM monitoring is used during VLP propagation, post purification, and periodically during storage. Some of the VLP preparations provide challenges for EM. The challenges include one or both of the following.
1. Visualizing the VLPs within extraneous cellular and VLP associated debris. 2. Visualizing the VLPs despite the effects caused by various chemicals used for preparation and purification. Details regarding EM results of the preparations along with methods for solving the difficulties encountered will be described in the presentation.

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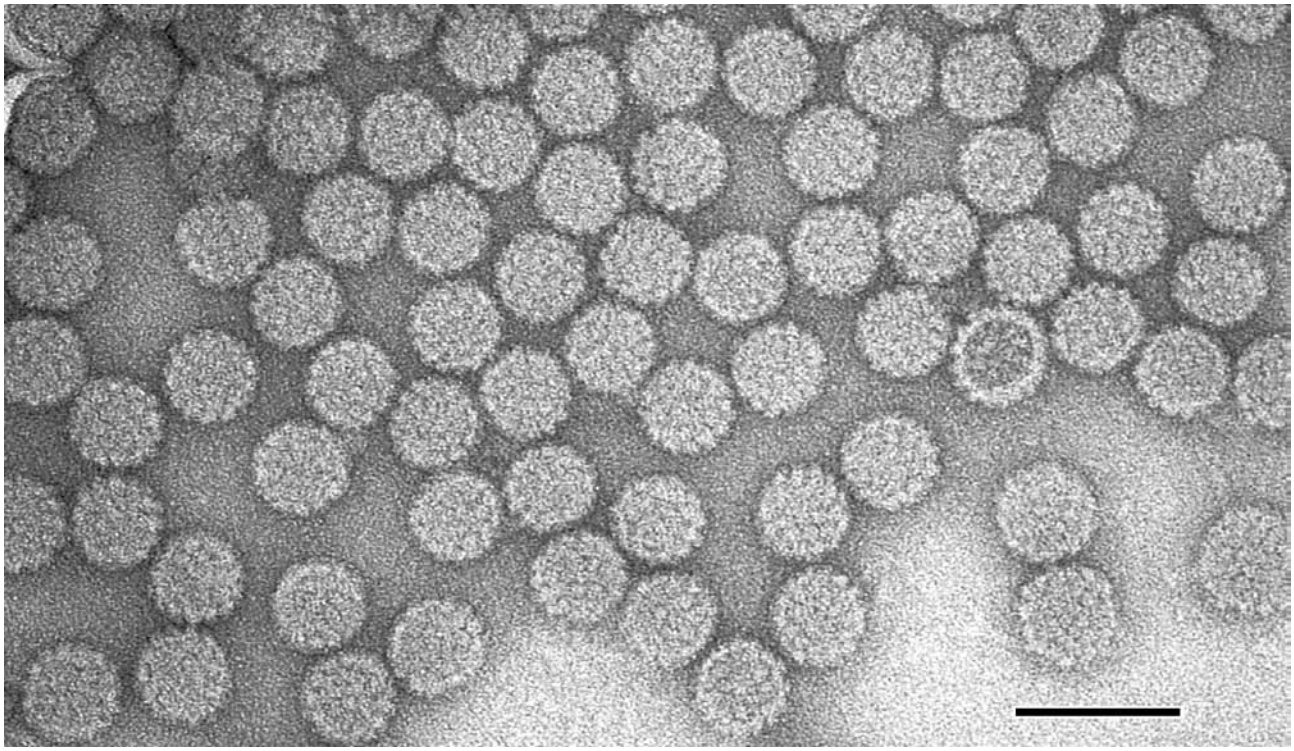

FIG. 1. HPV VLP 18. 45-60 nm HPV VLP L1 capsid protein is structurally similar to wild-type HPV. Bar represents 100 nm.

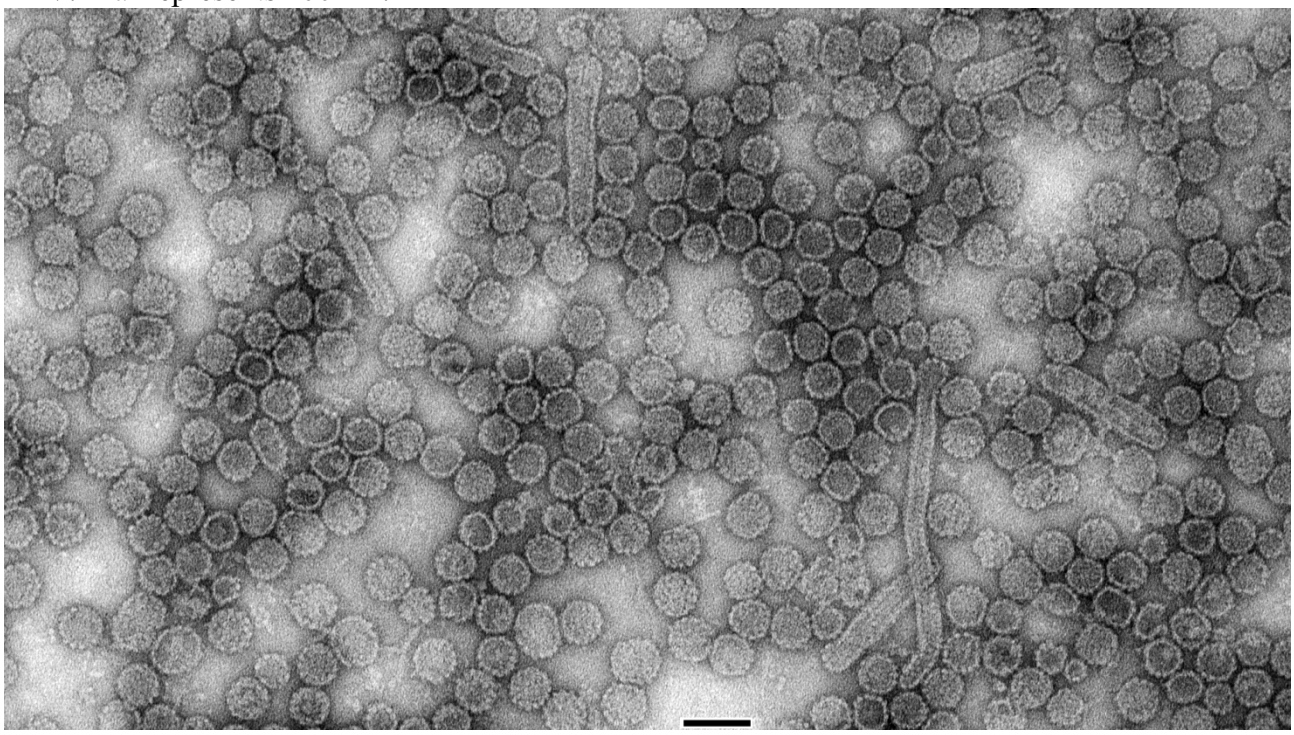

FIG. 2. HPV VLP 16. L1 capsid protein is similar to HPV 18 VLP capsid protein seen above. Numerous tubular forms of the capsid protein are interspersed among the VLP capsid proteins. Similar tubules are seen in preparations of wild-type papilloma-viruses and related polyomaviruses. Bar represents 100 nm.

https://doi.org/10.1017/S1431927611002315 Published online by Cambridge University Press