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Detection and quantitation of HPV in anogenital and oral tissues and fluids of HIV-positive individuals by real-time PCR

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Human papillomaviruses (HPV) remain a serious world health problem due to their association with anogenital and oral cancers and warts. While over 100 HPV types have been identified, only a subset is associated with malignancy. HPV16 and 18 are the most common oncogenic types, while HPV6 and 11 are the most common types responsible for anogenital warts. These four types cause up to 90% of HPV-associated disease. While other quantitative PCR (qPCR) assays can be used to detect oncogenic HPV, there is no single tube assay that distinguishes the most frequent oncogenic types and the most common types found in warts. A qPCR assay was developed that allowed for detection and quantitation of these 4 HPV types. Type-specific primer pairs and TaqMan probes allowed single tube multiplex reactions to be performed. Each HPV type was detected over a range from 2 H 10^1 to 2 H 10^6 copies/reaction, providing a reliable method of quantitating type-specific HPV. A Sybr Green-based qPCR assay was developed that utilizes degenerate primers targeting the E1 region of all HPVs. These assays were run in parallel with PCR/sequence gold standard on 76 oral cancers from HIV-negative individuals. Cervical and oral washes were collected from 25 HIV-positive women and 90 HIV-positive men, respectively, being screened for anogenital neoplasia. Samples were analyzed using the newly developed assays. Of the 115 samples, 16% were HPV positive. Cervical washes contained HPV types 44, 67, 35, and 68 and oral specimens contained HPV types 16, 11, 32, 6, 55, 73, and 70. These results indicate that these assays can be used to detect and quantitate HPV in clinical samples obtained by noninvasive measures.

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