# Detection and typing of human papillomaviruses combining different methods: PCR-RFLP, microarray and sequencing.

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Human papillomaviruses (HPV) are small oncogenic DNA viruses of which more than 200 types have been identified to date and about 40 infect the g tract. Although HPV infection is asymptomatic in most cases, persistent genital HPV infections can cause cervical cancer in women. Virtually all cervical cancer cases are caused by HPV, which is the most prevalent viral infection of the reproductive tract. Persistent cervical infection by specific HPV genotypes causes precursor lesions and cervical cancer. This discovery has revolutionized prevention strategies by directly targeting the causal agent. HPV genotyping tests have been shown to be relevant for screening to identify which HPV-positive women have persistent oncogenic HPV infection. Molecular tests has been considered promising strategies for primary screening, especially in older women who are at increased risk of developing cervical cancer. The aim of this study was to compare the performance of three different methodologies (RFLP-PCR, microarray and sequencing) for HPV genotyping. Three hundred twenty-five cervical samples were collected from sexually active women from November 2011 to March 2013. HPV were detected by PCR-multiplex using the consensus primers, PGMY0911 and PCO4/GH20 housekeeping gene. HPV-positive samples were typed by PCR-RFLP, microarray and sequencing. Thirty-two HPV different genotypes were identified by PCR-RFLP, microarray and sequencing and the most prevalent types were 16, 39, 53, 68 and 56. The multiplex-PCR proved to be useful to detect HPV. The PCR-RFLP showed good performance to identify up to two viral types in the same sample. The sequencing methodology proved to be an excellent tool for the identification of a single viral type, while the Papillocheck® was the best method for samples infected for more than two viral types. The PCR-RFLP has a high predictive negative value and can be used as a screening method. Complementary methodologies with high discriminatory power are necessary to identify samples with more than two viral types. Alternatives protocols like combination of a cheap in house PCR-RFLP and an expensive commercial methodology with high discriminatory power can be used, especially in places with low income to ensure best quality to screening.

**Keywords:** Human Papillomavirus, genotyping, PCR-RFLP, microarray and sequencing