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"Comparing Roche Linear Array® to Next Generation Sequencing for HPV Genotype Identification"

BACKGROUND: Human papillomavirus (HPV) is known to be a causative agent in most cervical neoplasia, thus identifying HPV in a patient's cervical swab is an important step in detecting risk for cervical cancer and preventing further development. The Linear Array Genotyping Test (LA) from Roche is a colorimetric reverse line blot hybridization array that detects 37 genotypes of HPV. The LA has been the standard assay used in research for HPV genotyping over the past two decades and has recently been discontinued. Newer high-throughput assays like Next Generation Sequencing (NGS) have broadened the variety of HPV types that can be detected. The use of MY09/11 degenerate primers allow for nonspecific amplification of HPV late gene 1(L1) regions and sequencing of amplicons through NGS. In this work the performance of NGS with MY09/11 primers will be compared to that of the LA.

METHODS: DNA extracts from cervical swabs, collected previously from patients in an HPV observational study, were utilized for this study. The samples were tested by the Linear Array Genotyping Test (Roche Diagnostics), according to manufacturer specifications. Next the samples were sequenced using the Illumina MiSeq platform and sequence reads aligned to HPV reference genomes using custom Perl scripts. Data from NGS was then compared to Linear Array results using Excel and Graphpad Prism.

RESULTS TO DATE: A total of 18 cervical samples were compared. Across the 18 samples, 23 HPV types were found by LA and 16 HPV types were found through NGS. Best concordance between LA and NGS was found when a read count threshold of ≥ 20 reads was applied to NGS output. There was an average concordance between LA and NGS of 92.01%. NGS found three HPV types not detected in these samples by LA, (HPV 33, 44, and 85). LA found HPV 35, 45, 51, 52, 55, 58, 68, 73, 84, and 89 infections that were not detected by NGS. No correlation was found between the number of mismatches between HPV genotype reference sequence and MY09/11 primers and the concordance of the type between LA and NGS results.

CONCLUSIONS TO DATE: NGS with MY09/11 primers were able to identify three genotypes not detected by linear array. While the platform identified unique HPV types, it detected fewer infections than the LA. Cross-hybridization of LA hybridization probes has been reported in the literature and may explain the discordance of HPV detection between assays. Overall, the NGS platform circumvents the cross-hybridization of LA as well as allows for the detection of a broader range of HPV types.