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## "Single vs. Six Marker DNA Methylation Panel for Detection of Cervical Dysplasia"

BACKGROUND: Cervical cancer screening with primary high-risk Human Papillomavirus (hrHPV) testing has high sensitivity but low specificity for women with high-grade cervical intraepithelial neoplasia (CIN 3). A specific screening method to determine which hrHPV positive women should be referred for colposcopy and possible treatment is urgently needed. DNA Methylation analysis offers a promising tool for this use. GynTect® (oncgnostics GmBH) is a real-time PCR (rtPCR) based DNA methylation assay that uses residual cytology samples and is designed to predict the histopathology of a woman's cervical biopsy. It tests for methylation in promoter regions of six genes: ASTN1, DLX1, ITGA4, RXFP3, SOX17, and ZNF671. A recent study found methylation analysis of ZNF671 alone to perform well in detection of cervical dysplasia. We aim to study the performance of all six Gyntect markers versus the performance of ZNF671 methylation alone for detection of cervical dysplasia.

METHODS: Residual fluid of Papanicolaou tests collected in New Orleans, LA were tested for HPV status via PCR amplification of the L1 gene and gel electrophoresis. Residual fluid was then bisulfite treated and tested with the Gyntect assay according to manufacturer specifications. The results of the Gyntect assay were then analyzed for positivity of all six markers vs positivity of ZNF671 alone and compared to cervical biopsy histopathology results.

RESULTS TO DATE: 21 samples with complete demographic, HPV, pathology, and methylation testing data were included. Average patient age was 41 (range 26-64). Patients were 66.7% Black/African American, 19.0% White, and <5% Hispanic/Latino. 47.6% of samples were hrHPV positive. Cervical biopsy results were 57.1% negative, 19.0% CIN 1, 4.7% CIN 2, and 14.3% CIN 3. 52.4% of Gyntect assays run were deemed valid. Of the valid assays, both Gyntect and ZNF671 alone were positive for 100% of CIN2 and CIN 3 cases (4/4). Of valid assays, there was 100% concordance between Gyntect and ZNF671 for both positive and negative results.

CONCLUSIONS TO DATE: These results show promising concordance between results of the entire Gyntect six marker panel, and the single marker ZNF671. Use of this single marker could reduce both cost for patients and complexity for labs using DNA methylation as a triage test for hrHPV cervical cancer screening. There are limitations to this study, including small sample size, and high rate of invalid Gyntect results. These invalid assays could be due to sample degradation, or low cell count in residual cytology fluid. Future studies including a larger sample size and samples collected specifically for DNA methylation testing could show a higher rate of valid assays.