

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

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Introduction

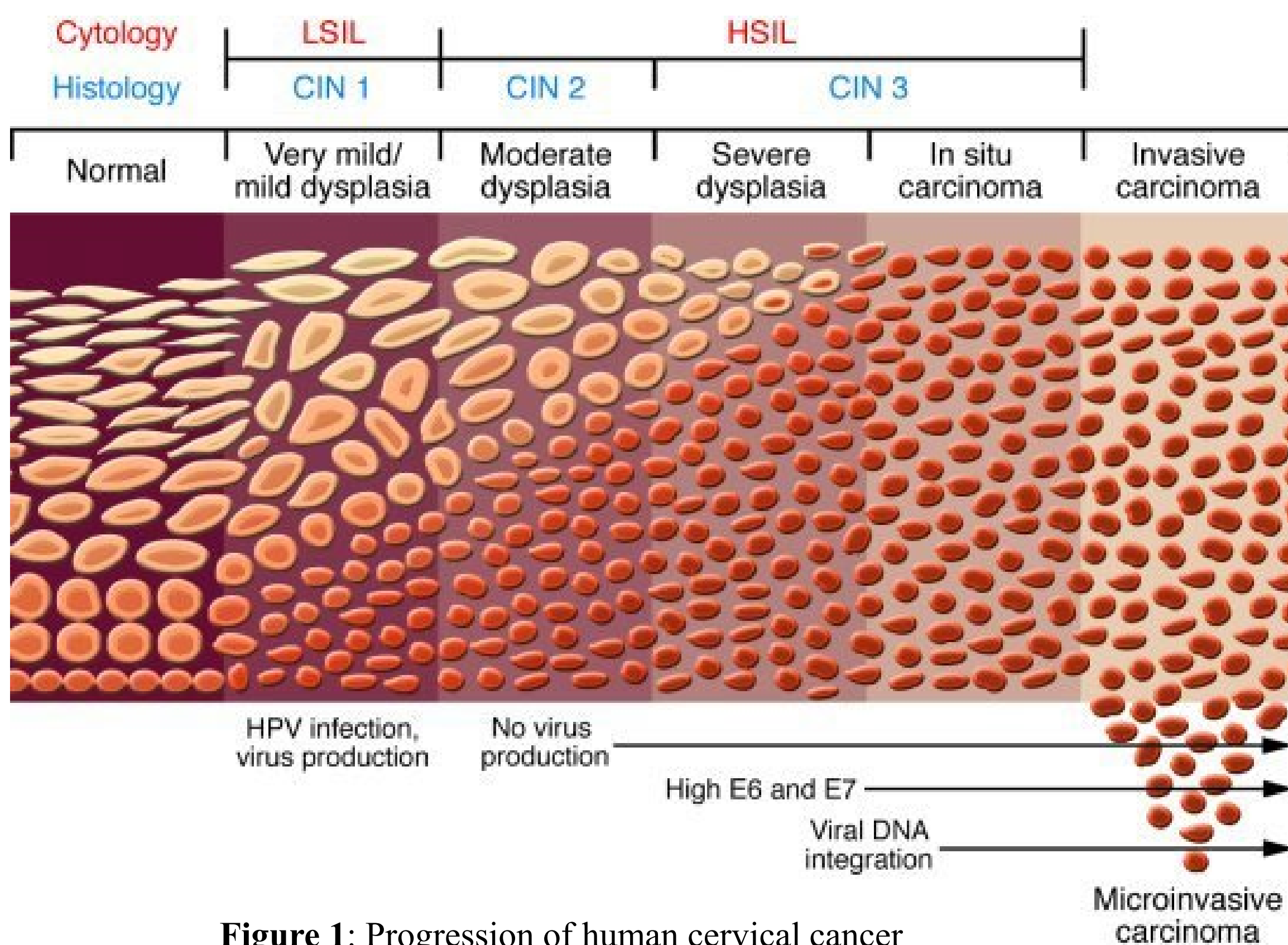


Figure 1: Progression of human cervical cancer after HPV infection¹

- Persistent infection with high-risk Human Papillomavirus (hrHPV) is a known cause of cervical dysplasia.²
- Primary screening for cervical dysplasia with hrHPV testing is gaining favor, offering excellent sensitivity, but lacking specificity to discern transient hrHPV infection from the persistent infection needed to cause cervical dysplasia.²
- A highly specific test is needed to triage women positive for hrHPV and prevent unnecessary colposcopy and treatment.

Gyntect Gene Targets	Name	Function	Points if Positive
ASTN 1	Astroactin 1	Neuronal adhesion molecule ⁶	2
DLX1	Distal-less homeobox 1	Transcription factor ⁷	1
ITGA4	Integrin subunit alpha 4	Alpha chain family of cell adhesion and signaling protein Integrin ⁸	2
RXFP3	Relaxin family peptide receptor 3	G-protein coupled receptor for the relaxin family peptides ⁹	2
SOX17	SRY box transcription factor 17	Transcription factor ¹⁰	2
ZNF671	Zinc finger protein 671	Transcriptional repressor that is a member of the family of Kruppel-associated box zinc finger proteins (KRAB-ZFPs) ¹¹	6

Table 1: Name and function of genes targeted by Gyntect methylation analysis.⁴ Score ≥ 6 is read as Gyntect being Positive².

- DNA methylation is an epigenetic event that functions in transcriptional repression, and certain genes have been found to be highly methylated in cervical cancer specimens.³
- Gyntect® (oncnostics GmbH) is a DNA methylation-based assay that detects hypermethylation in the promoter regions of 6 genes to predict cervical dysplasia on a molecular basis.⁴
- Gyntect performs well triaging primary hrHPV screen positive women, with sensitivity between 31.6-67.7% for Cervical Intraepithelial Neoplasia 3 (CIN3) and above, and specificity between 82.6-95.9% for < CIN3.⁴
- Recently, methylation analysis of ZNF671 alone has shown promising performance in triage of hrHPV positive women, achieving sensitivity 79.5% for CIN3+ and specificity 79.8% for < CIN3, in a cohort of 281 women.⁵

Objective

The objective of this work is to compare the performance of all six Gyntect markers versus the performance of ZNF671 methylation alone for detection of cervical dysplasia.

Patient Characteristics

Characteristic	Overall (n = 21)	Gyntect Valid (n = 11)	Gyntect Positive (n = 7)
Age			
Mean	41.2	39.0	37.6
Race/Ethnicity			
Black/African American	14 (67.7%)	10 (71.4%)*	6 (42.9%)
White/Caucasian	4 (19.0%)	0 (0.0%)	0 (0.0%)
Other	3 (14.3%)	1 (33.3%)	1 (33.3%)
Relationship Status			
Single	15 (71.4%)	9 (60.0%)	6 (40.0%)
Cohabiting With Partner	2 (9.5%)	0 (0.0%)	0 (0.0%)
Married	2 (9.5%)	1 (50.0%)	0 (0.0%)
Divorced/Separated	2 (9.5%)	1 (50.0%)	1 (50.0%)
HPV Status			
Positive	10 (47.6%)	3 (30.0%)	3 (30.0%)
Negative	11 (52.4%)	8 (72.2%)	4 (36.4%)
Histopathology			
Negative	12 (57.1%)	4 (33.3%)	2 (16.7%)*
CIN 1	4 (19.0%)	3 (75.5%)	1 (25.0%)*
CIN 2	1 (4.8%)	1 (100.0%)	1 (100.0%)*
CIN 3	4 (19.0%)	3 (75.0%)	3 (75.0%)*

Table 2: Patient characteristics for the overall study group, Gyntect valid tests, and Gyntect valid tests. Statistically significant results marked in red, detailed in Results.

1 vs 6 Marker Analysis

Methylation Analysis Result vs Histopathology

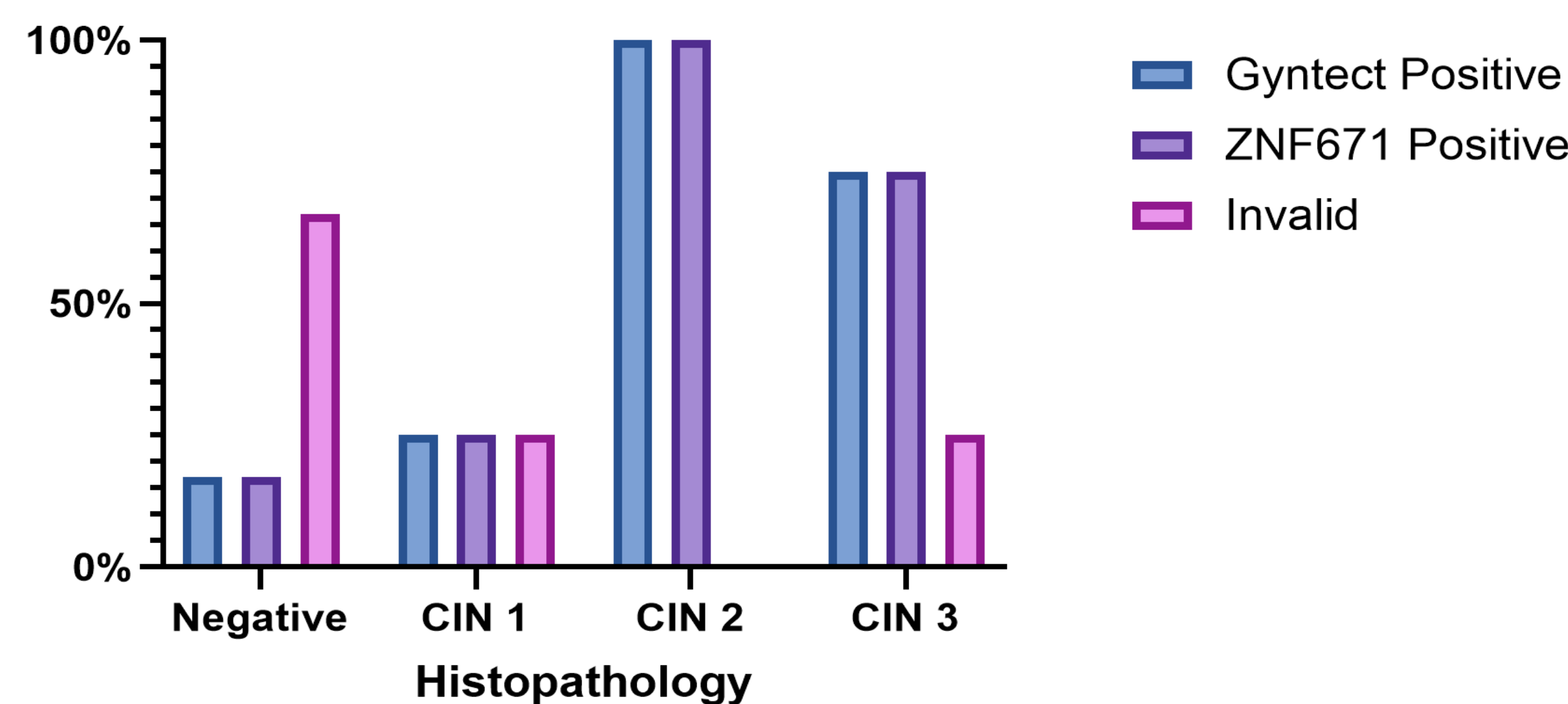


Figure 2: Within each histopathological group, the percent of the group with each methylation result.

Methods

- Residual fluid of Papanicolaou tests collected in New Orleans, LA were tested for HPV status via PCR amplification of the L1 gene, gel electrophoresis, and genotyped by the Linear Array Genotyping Test (Roche Diagnostics), according to manufacturer specifications.
- 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.
- Demographic data including age, race/ethnicity, and relationship status was gathered from surveys completed at the time of sample collection.
- Histopathology results were collected from medical records.

Results

- 52.4% (11/21) of Gyntect tests were scored as valid within the manufacturer's parameters for Cp and melting temperature values.
- There were significantly more Black/African American individuals with valid tests compared to other races/ethnicities ($p = 0.0134$).
- When comparing Gyntect valid to invalid tests, there was no significant difference in age, relationship status, HPV status, histopathology, or sample nanodrop DNA concentrations between the two groups.
- There were significantly more Gyntect negative tests among negative samples and low grade lesions (< CIN 2) than high grade lesions (\geq CIN 2) ($p = 0.0251$).
- Among valid tests, for \geq CIN 2, Gyntect and ZNF671 methylation analysis had a sensitivity of 100.0% (95% CI 51.0% to 100.0%) and specificity of 57.1% (95% CI 25.05% to 84.2%).
- Among valid tests, there was 100% concordance between results of the whole Gyntect six marker panel, and ZNF671 alone.

Conclusions

- Six marker and ZNF671 single marker Gyntect results are highly concordant.
- Preliminary sensitivity is higher and specificity lower than previous data.
- Limitations
 - Small sample size
 - Frequent invalid test results possibly due to:
 - Sample degradation
 - Low cell count in cytology fluid
 - PCR inhibitors

- Use of this single marker test could reduce both cost for patients and complexity for labs using DNA methylation as a triage test for primary hrHPV cervical cancer screening.

Future Directions

- We are currently in the process of beginning a cross-sectional cohort study of women presenting to colposcopy clinic after a positive cervical cancer screening test. We are hoping to begin collecting samples for Gyntect and ZNF671 methylation analysis soon.
- We have begun writing a literature review on the topic of DNA methylation analysis for detection of cervical dysplasia, the landscape of currently available assays, and the genes that they test for.

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