FISH-Based HPV-Associated Cancer Test (FHACT™)

Overview
Cervical infection with high-risk human papillomavirus (hrHPV) is the necessary cause of cervical cancer. Secondary prevention of the disease relies on the accurate identification and treatment of high-grade precursors to prevent progression to invasive disease. Inclusion of hrHPV typing tests in cytology-based screening programs has clearly improved the sensitivity with which precancerous lesions are detected. Indeed, women testing hrHPV-negative have an extremely low risk of cancer over 3-5 years. The management of women testing hrHPV-positive in the setting of a normal cytology or abnormal cells of undetermined significance (ASC-US) however, is much less clear. About 90% of HPV infections will be cleared within 1-2 years and only a minority of precancerous lesions will progress to a higher grade lesion (5-year risk of progression is reported to be about 4-6%). Women diagnosed with a low grade squamous intraepithelial lesion (LSIL) have a marginally higher risk of progression but hrHPV testing does allow any additional benefit in risk assessment since most are hrHPV-positive. It is in these clinical settings affecting over 2 million women per year in the US, that integration of additional biomarkers in the triage process is much needed in order to limit unnecessary colposcopies and excisional procedures and identify those women with lesions that are at risk of progression to a higher grade.

Associated with such progression is the appearance of host oncogenic events, such as non-random chromosomal abnormalities including genomic gain [1]. The FISH-Based HPV-Associated Cancer Test (FHACT™) assesses these genomic alterations. Gain of 3q26 (TERC) has been detected with increasing frequency in cervical lesions with increasing severity and ultimately is observed in about 75% of cervical cancers [2-5]. Gain of 5p15, 20q13, and chromosome 7 share a similar pattern of appearance in precancerous cytology specimens by FISH but with lower frequencies, consistent with their lower occurrence in cervical cancer (40-45% for 5p15 and 20q13, and 15% for chromosome 7) [6-8]. Gain of any one of the four loci is detected in up to 90% of all cervical cancers [1].

Clinical Indications
High-risk HPV-positive normal or ASC-US, and LSIL cytology specimens.

Clinical Utility
Identifies specimens with clonal chromosomal aberrations commonly detected in cervical cancer and that are observed with higher frequencies in cervical lesions with increasing cytologic severity.

Methodology and Interpretation
Fluorescence in situ hybridization (FISH) analysis is performed on nuclei of cells derived from a liquid pap specimen, using the FHACT™ combination probe (CGI Italia): 3q26 (TERC) (red), 5p15 (D5S2095) (green), 20q13 (D20S911) (gold) and CEP7 (aqua). The assay has been optimized to identify any copy gains of 3q26, 5p15, CEP7 and 20q13. FHACT™ results should be used in conjunction with cytological findings.

Assay Specifications

Reporting
A minimum of 2,000 cells are analyzed. Probe pattern of 3 or more signals of any probe is considered a gain. If the number of cells with the abnormal signal pattern exceeds the established normal cutoff value for a probe, then the result is reported as a positive result. A negative result does not preclude the presence of an abnormal clone.

TAT
5-7 days

CPT Codes
88271(4); 88275(1); 88291

Specimen Requirements
- Liquid cytology in PreservCyt or SurePath.
- Stored at 4°C and transported on ice packs within 24 hours after collection.

CGI Laboratory Licensure
CAP (Laboratory #: 7191582, AU-ID: 1434060), CLIA (Certificate #: 31D1038733), New Jersey (CLIS ID #: 0002299), New York State (PFI: 8192), Pennsylvania (031978), Florida (800018142), Maryland (1395).
## FISH-Based HPV-Associated Cancer Test (FHACT™) Sample Report

**Results:**

<table>
<thead>
<tr>
<th>PROBE SETS</th>
<th>CHROMOSOME LOCIS</th>
<th># CELLS ANALYZED</th>
<th>ABNORMAL CUTOFF VALUE (95% CI)</th>
<th>RESULTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>3q26 (TERC)</td>
<td>2000</td>
<td>125</td>
<td>≥ 6%</td>
<td>Abnormal Signal Pattern</td>
</tr>
<tr>
<td>5p15 (DSS2095)</td>
<td>2000</td>
<td>125</td>
<td>≥ 2%</td>
<td>Abnormal Signal Pattern</td>
</tr>
<tr>
<td>20q13 (D20S911)</td>
<td>2000</td>
<td>---</td>
<td>≥ 2%</td>
<td>Normal Signal Pattern</td>
</tr>
<tr>
<td>CEP7</td>
<td>2000</td>
<td>125</td>
<td>≥ 4%</td>
<td>Abnormal Signal Pattern</td>
</tr>
</tbody>
</table>

**ISCN Nomenclature:**

nuc ish(3q26x4,5p15x3,20q13x2,CEP7x4)[125/2000]

**Summary:**

Positive for gain of 3q26, 5p15 and CEP 7

**Interpretation:**

The FISH analysis showed abnormal signal patterns for gain for 3q26, 5p15 and CEP7 in 6.3% cells.

Carcinogenic types of human papillomavirus (HPV) are a necessary cause of cervical cancer but many HPV-positive women will clear their HPV infection without developing cancer. Thus, HPV-DNA based tests have limited specificity to identify HPV-positive women who are at risk of progressing to high-grade disease and cancer. Associated with such progression is the appearance of host oncogenic events, such as non-random chromosomal abnormalities including genomic gain [1]. Gain of 3q26 has been detected with increasing frequency in cervical lesions with increasing severity by fluorescence in situ hybridization (FISH) and ultimately is observed in about 75% of cervical cancers [2-5]. Gain of 5p, 20q, and chromosome 7 share a similar pattern of appearance in precancerous cytology specimens by FISH but with lower frequencies, consistent with their lower occurrence in cervical cancer (40-45% for 5p and 20q, and 15% for 7) [6-8]. Gain of any one of the four loci is detected in up to 90% of all cervical cancers [1].

Fluorescence in situ hybridization (FISH) analysis was performed on nuclei of cells derived from a liquid pap specimen, using the FHACT™ combination probe (CGI Italia): 3q26 (TERC) (red), 5p15 (DSS2095) (green), 20q13 (D20S911) (gold) and CEP7 (aqua). The assay has been optimized to identify any copy gains of 3q26, 5p15, CEP7 and 20q13. Probe pattern of 3 or more signals of any probe is considered a gain, if the number of cells with the abnormal signal pattern exceeds the established normal cutoff value. The result is recorded as a positive result. A negative result does not preclude the presence of an abnormal clone.

**References:**