

**FINAL REPORT**

**Patient:**  
**Sex:**  
**DOB:**  
**Client:**  
**Provider:**

**Specimen ID:**  
**Collected:**  
**Received:**  
**Reported:**  
**Specimen Type:**

**TISSUECYPHER® RISK SCORE AND RISK CLASS**

\*RISK SCORE: **3.2** (range 0 – 10)

RISK OF CLASS: **LOW**

5-Year Probability of Progression: **2%**

\*If multiple specimens were submitted for testing, the reported result is based on the highest scoring specimen.

**CLINICAL EXPERIENCE**

This test is indicated for patients diagnosed with non-dysplastic (ND), indefinite for dysplasia (IND) or low grade dysplasia (LGD) Barrett's esophagus. Risk of progression to high grade dysplasia or esophageal adenocarcinoma within five years was determined from a multi-institutional nested case-control validation study involving 366 patients with Barrett's esophagus from four institutions<sup>1</sup>. A total of five confirmatory clinical performance studies have been completed and published<sup>1-5</sup>. The clinical utility and cost-effectiveness have also been demonstrated in published studies<sup>6-7</sup>.

The results provided here are adjunctive to the ordering physician's workup for patients with Barrett's esophagus. The reported 5-year probability of progression was adjusted based on estimated prevalence as described in the validation study<sup>1</sup>, however, the prevalence of progression of Barrett's esophagus may vary between clinical institutions.

**TISSUECYPHER BARRETT'S ESOPHAGUS ASSAY DESCRIPTION**

The test uses whole slide digital images from formalin-fixed paraffin-embedded (FFPE) tissue sections from endoscopic biopsy specimens. Using a proprietary artificial intelligence-driven quantitative algorithm, a risk score for progression to high grade dysplasia or esophageal adenocarcinoma is generated from the image analysis results. The risk score ranges from 0-10, with 0 indicating lowest risk and 10 indicating highest risk, and patients are classified as low, intermediate or high-risk for progression to high grade dysplasia or esophageal adenocarcinoma within five years. The risk classes provide predictive power (hazard ratio=7.3, p<0.0001) that is independent of clinical and pathologic features of age, sex, segment length, pathologic diagnosis (ND, IND or LGD) and p53 as a single biomarker<sup>1</sup>.

The TissueCypher Barrett's Esophagus Assay is a multi-analyte assay with algorithmic analysis that uses automated image analysis to objectively quantify the expression and localization of nine biomarkers (p16, p53, alpha-methylacylCoA racemase [AMACR], HER2/neu, Cytokeratin-20 [K20], Cyclooxygenase-2 [COX-2], CD68, Hypoxia-inducible factor 1- $\alpha$  [HIF1A], and CD45RO) in the context of tissue morphology<sup>8</sup>.

For additional information about the development and validation of the TissueCypher Barrett's Esophagus Assay, visit the Products tab of [www.castlebiosciences.com](http://www.castlebiosciences.com).

This test was developed, and its performance characteristics determined by Castle Biosciences, Inc. It has not been cleared or approved by the FDA. The laboratory is regulated under CLIA as qualified to perform high-complexity testing. This test is used for clinical purposes. It should not be regarded as investigational or for research. Patent Pending.

*Based on my review, the TissueCypher® Barrett's Esophagus Assay batch controls passed quality assessment and the observed biomarker expression patterns are consistent with the results described herein.*

---

Castle Biosciences, Inc.

Sample

#### References

1. Critchley-Thorne et al. Cancer Epidemiol Biomarkers Prev 2016;25(6):958-68. A Tissue Systems Pathology Assay for High-Risk Barrett's Esophagus. 2. Critchley-Thorne et al. Cancer Epidemiol Biomarkers Prev. 2017;26(2):240-248. A Tissue Systems Pathology Test Detects Abnormalities Associated with Prevalent High-Grade Dysplasia and Esophageal Cancer in Barrett's Esophagus. 3. Davison, et al. Am J Gastroenterol 2020;115:843-852. Independent Blinded Validation of a Tissue Systems Pathology Test to Predict Progression in Patients With Barrett's Esophagus. 4. Frei, et al. Clin Transl Gastroenterol 2020;11. Independent Validation of TissueCypher to Predict Future Progression in Non-Dysplastic Barrett's Esophagus: A Spatial-Temporal Analysis. 5. Frei, et al. Am J Gastroenterol 2021;116:675-682. Tissue Systems Pathology Test Objectively Risk Stratifies Barrett's Esophagus Patients With Low-Grade Dysplasia. 6. Hao, et al. Clinicoecon Outcomes Res. 2019 Oct 25;11:623-635. A Cost-Effectiveness Analysis Of An Adenocarcinoma Risk Prediction Multi-Biomarker Assay For Patients With Barrett's Esophagus. 7. Diehl, et al. Endosc Int Open. 2021 Mar;9(3):E348-E355. TissueCypher Barrett's esophagus assay impacts clinical decisions in the management of patients with Barrett's esophagus. 8. Prichard et al. J Pathol Inform. 2015 Aug 31;6:48. TissueCypher™: A systems biology approach to anatomic pathology.

This test was developed, and its performance characteristics determined by Castle Biosciences, Inc. It has not been cleared or approved by the FDA. The laboratory is regulated under CLIA as qualified to perform high-complexity testing. This test is used for clinical purposes. It should not be regarded as investigational or for research. Patent Pending.