The testing process to generate the TissueCypher Barrett’s Esophagus risk score and risk class are summarized below**:**

*Multiplexed immunofluorescent labeling and whole slide scanning:* Five-micron sections of FFPE biopsies were auto-labeled by multiplexed immunofluorescence for p16, alpha-methylacyl-CoA racemase (AMACR), p53, HER2, cytokeratin-20 (K20), CD68, COX-2 (cyclo-oxygenase-2), HIF-1 (hypoxia-inducible factor 1-alpha subunit), and CD45RO, with Hoechst 33342 labeling of nuclei, according to previously described methods (1-3) using the BOND Rx (Leica Biosystems, Inc., Buffalo Grove, IL). Labeled tissue slides were digitized using a standard operating procedure at 20x magnification on ScanScope FL scanners (Leica BioSystems) as previously described (1).

*Quantitative image analysis and generation of risk scores:* Whole slide fluorescence images were analyzed using the TissueCypher® Image Analysis Platform (Cernostics, Inc., Pittsburgh, PA) to extract the 15 pre-defined features (quantitative measures of protein- and morphology-based biomarkers) that are the required input for the risk classifier, as described previously (1, 2, 4). Risk scores (0-10) and risk classes (low-, intermediate- and high-risk) were calculated using the 15 quantitative measures, as previously described (2, 4).

**References**

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