Superior Determination of HPV using RNAscope™ *In Situ* Hybridization

HPV has been identified as a causal agent in oropharyngeal squamous cell carcinoma (OPSCC) with the incidence of HPV-related OPSCC increasing dramatically over the last several decades. As patients with HPV-positive OPSCC have a significantly better survival compared to patients with HPV-negative OPSCC, testing for HPV status is critical.

The RNAscope™ RNA *In Situ* Hybridization Platform, including chromogenic detection reagents and target-specific probes for both High Risk and Low Risk HPV types, provides clear and unequivocal assessment of HPV status in FFPE tissue. (Figure 1) In numerous peer-reviewed publications, the RNAscope HPV Detection Reagents have demonstrated significantly higher specificity and equivalent sensitivity in direct comparisons with p16 IHC for the presence of high-risk HPV in OPSCC.

The combination of high sensitivity and high specificity ensures ready identification of HPV-related tumors while reducing potential false positives that can result from the use of surrogate markers of HPV such as p16. Unlike p16 IHC, RNAscope allows the pathologist to interrogate any single HPV type or combination of HPV types.

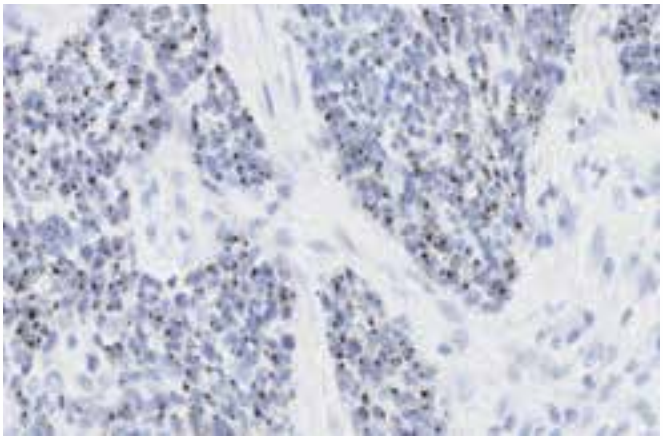


Figure 1: RNAscope Probe HPV HR18 (cocktail including HPV types 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, & 82)
Tissue: Oropharyngeal squamous cell carcinoma (OPSCC)

Higher Specificity Increases Confidence

Common techniques for HPV detection in OPSCC, including DNA ISH and p16 IHC, often lead to false positives or false negatives. RNAscope gives diagnostic pathologists a direct method for detection of active HPV through interrogation of E6/E7 viral oncogene mRNA, targets that provide unequivocal evidence of HPV in contrast to p16, a surrogate marker, which is upregulated by HPV as well as other oncogenic pathways. (Figure 2) The presence of HPV E6/E7 mRNA is considered by experts to be the gold standard for determination of HPV status in OPSCC.[1,2] (Figure 3)The high degree of specificity and sensitivity provides optimal classification of HPV-status in tumors, thereby ensuring that patients are given the correct treatment options and prognostic information.[3]

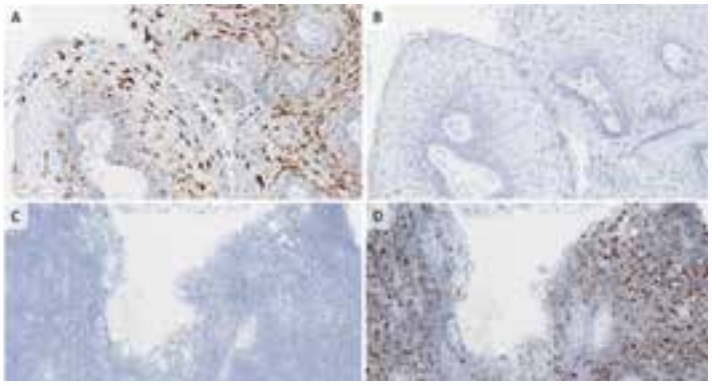


Figure 2:
A and C Probe: RNAscope Probe HPV LR6 (cocktail including HPV types 6,11, 40, 42, 43, & 44)
B and D Probe: RNAscope Probe HPV HR18
A and B Tissue: Cervical Condyloma
C and D Tissue: Ororpharyngeal squamous cell carcinoma

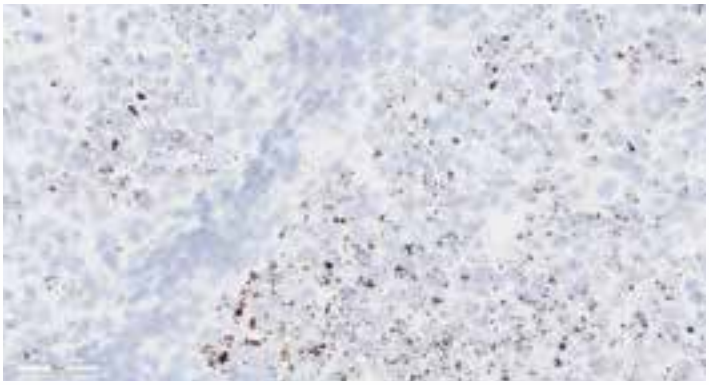


Figure 3: HPV 16 + 18
Tissue: Oropharyngeal squamous cell carcinoma (OPSCC)

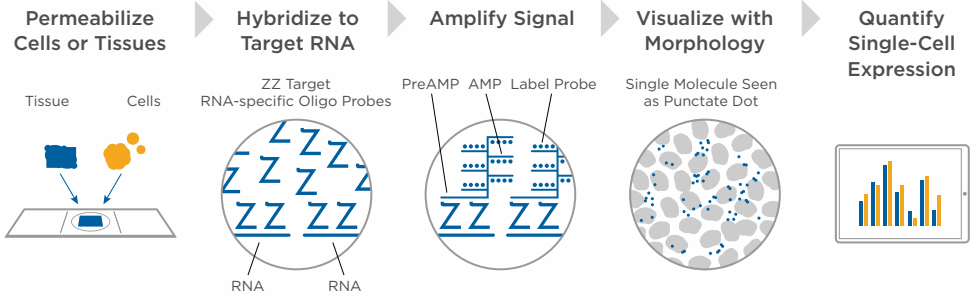
Comparison of Tissue-Based Tests for HPV Detection in OPSCC

Test	Target	Evidence of HPV Activity	Sensitivity	Specificity
IHC	p16	-	+++ (97%)	+ (86%)
DNA ISH	HPV DNA	-	+ (83%)	++ (89%)
RNAscope (RNA ISH)	E6/E7 mRNA	+	+++ (97%)	+++ (96%)

Schache et al. *Br J Cancer* (2013); Gao et al. *Int J Cancer* (2012); Mirghani et al. *Mod Path* (2015)

RNAscope Brings Molecular Detection to Anatomic Pathology

The RNAscope *in situ* Hybridization Platform, including detection reagents and target-specific probes, is a robust technology that allows for the identification of RNA expression patterns and localization at the single cell level with spatial and morphologic context. RNAscope is highly sensitive and specific due to its double Z probe design, resulting in an extremely high signal-to-noise ratio of staining in FFPE tissues relative to traditional RNA ISH, allowing diagnostic pathologists to visualize, localize, and quantify biomarker expression simultaneously. The technology is readily available on automated staining platforms, including the Leica Bond III, for ease of use, high reproducibility, and seamless fit into the anatomic pathology lab workflow. Furthermore, RNAscope provides labs with the opportunity to add new diagnostic biomarkers to their menus and provide better options for problematic IHC tests.



The technology is readily available on automated staining platforms for ease of use, high reproducibility, and seamless fit into the anatomic pathology lab workflow.

References: 1. Mills, et al 2018 Am J Surg Pathol **2.** NCCN Guidelines 2018 v1.2018 **3.** Craig, et al 2019 Br J Cancer

Learn more about RNAscope and ACD ASR and GPR products at: https://acdbio.com/diagnostics
To request additional information or a quote, contact: acd_clinical@bio-techne.com

7707 Gateway Boulevard, Newark, CA 94560 1.510.576.8800 (Main) | 1.877.576.3636 (Toll Free)