Background:
- High-risk human papillomavirus (HR-HPV) infection is a major etiologic agent in a subset of head and neck squamous cell carcinomas (HNSCCs), and its recognition has prognostic and predictive implications.
- The availability of a sensitive and specific test to assess HR-HPV status is limited.
- We evaluate an RNA in situ hybridization (ISH) method using branch chain technology to detect HR-HPV (Affymetrix, Santa Clara, CA) and compare its results with DNA ISH, p16 immunohistochemistry, and PCR.

Design:
- We reviewed clinico-pathologic details for a cohort of 54 patients with HNSCC.
- All cases were stained with a manual RNA ISH assay ViewRNA™ technology (Affymetrix, Santa Clara, CA), which detects HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 66), an automated DNA ISH assay which detects the same HR-HPV types, and an immunohistochemical assay for p16.
- An automated RNA ISH method was also developed, and a subset (84%, n=45) of cases were repeated on this platform. Most cases (83%, n=45) were also tested with a RNA ISH assay that detects only HR-HPV types 16 and 18.
- PCR based assays were used for adjudication.

Principle of RNA ISH (Affymetrix, Santa Clara, CA)

Results
- The majority of HNSCC cases were interpretable by the ISH assays: 96% (n=52) for RNA-ISH and 94% (n=51) for DNA-ISH.
- Sixty three percent (n=34) of samples were positive for HR-HPV using the manual RNA ISH platform, while 39% (n=21) were positive using the DNA-ISH platform.
- The automated RNA ISH platform performed similarly to the manual version, and the 2 platforms showed 96% concordance (n=43/45 cases).

Take Home Message:
- RNA-ISH showed strong, easily interpretable signals in 96% of the cases tested.
- The automated version of the RNA ISH assay demonstrates high concordance with the manual version.
- The automated version of the RNA-ISH assay is easily implementable into the workflow of the diagnostic pathology laboratory.
- RNA ISH assay is more sensitive than DNA ISH assay.

Conclusion:
- The branch chain RNA-ISH assay is as specific as DNA-ISH for the detection of HR-HPV in HNSCC but is more sensitive when each is compared with p16 and PCR testing.
- HPV 16 and 18 represented the most common viral types detected.
- Overall, our results support that RNA-ISH appears to be a sensitive and specific method for the detection of transcriptionally-active HR-HPV in HNSCC.