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Supplemental Appendix for:
De-Escalated Adjuvant Therapy after Transoral Robotic Surgery for HPV related Oropharyngeal Carcinoma: The Sinai Robotic Surgery (SIRS) Trial
Brett A. Miles et al.

Appendix S1. HPV Testing Specifics

Immunohistochemistry for p16 was performed using a mouse monoclonal antibody (MTM Laboratories, Heidelberg, Germany) and staining was visualized using the Ultra view polymer detection kit (Ventana Medical Systems, Tucson, AZ) on a Ventana BenchMark XT autostainer (Ventana). Per established diagnostic protocols, only those cases that demonstrated diffuse staining (strong nuclear and cytoplasmic positivity in >70% of tumor cells) were reported as positive for p16 overexpression.(10)

For HPV PCR testing, the Maxwell 16 FFPE Tissue LEV DNA Kit (Promega, Madison, Wisconsin) was used for DNA extraction from tissue sections. Real-time PCR was performed with Roche Light cycler 480 and a High-Resolution Melting Master kit (Roche, Indianapolis, IN) using PCR primers (GP5+ and GP6+) specifically targeted to L1 region covering more than 31 HPV types found in cervical and head and neck cancer. Once the initial screening for HPV DNA was confirmed, type- specific primers and probes for HPV 16 or HPV 18 (targeting E6 region) were used to detect HPV 16 or HPV 18 by real time PCR. In the event HPV16/18-specific PCR was negative, Sanger Sequencing was performed to detect other HPV genotypes after amplification. Regardless of serotype, confirmation of HPV+ allowed enrollment into the trial.