Appendix 2 (as supplied by the authors): HPV testing procedures

Immunohistochemistry was performed on formalin-fixed paraffin-embedded tissue using various p16 antibodies at each site: the CINtec p16INK4A Histology Kit (REF# 9517; MTM Laboratories Westborough, MA) was used at both the Princess Margaret Cancer Centre [1] and the Nova Scotia Health Authority; Clone JC8, Novus Biologicals, Littleton, CO was used at the Tom Baker Cancer Centre [2]; and the CINtecp16 histology detection kit by VENTANA (Ventana Medical Systems, Inc. Tucson, Arizona, USA) was used at the Cross Cancer Institute. p16 status was determined semi-quantitatively as none, weak, strong focal, or strong diffuse [1]. If weak or focal p16 staining was observed, this was confirmed by polymerase chain reaction (PCR) using the GenPoint Amplified Signal Detection System (DakoCytomation, Carpinteria, CA) at the Princess Margaret Cancer Centre only [1]; PCR was not used elsewhere. Two pathologists independently rated the degree of semi-quantitative p16 immunostaining at one centre, observing 100% agreement [2]. p16 status was dichotomized as positive (p16+) if staining was strong diffuse or confirmed by PCR, and negative (p16-) otherwise.

References

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2. Lau HY, Brar S, Klimowicz AC, et al. Prognostic significance of p16 in locally advanced squamous cell carcinoma of the head and neck treated with concurrent cisplatin and radiotherapy. *Head Neck* 2011;33:251-6.

Appendix to: Habbous S, Chu KP, Lau H, et al. Human papillomavirus in oropharyngeal cancer in Canada: analysis of 5 comprehensive cancer centres using multiple imputation. *CMAJ* 2017. DOI:10.1503/cmaj.161379.