# Human papillomavirus testing in diagnostic head and neck histopathology

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## Abstract

Assessment of human papillomavirus (HPV) status is a requirement for the diagnosis of HPV-associated oropharyngeal squamous cell carcinoma (OPSCC) and metastatic squamous cell carcinoma in cervical lymph nodes where the location of the primary neoplasm is unknown. Within the diagnostic histopathology laboratory, there should be a validated and reproducible HPV testing strategy that can provide HPV status within a reasonable timeframe to inform patient care. Although these requirements are recognized by the head and neck oncology community, there is no internationally accepted standard for HPV testing. A two-tiered approach incorporating p16 immunohistochemistry with specific HPV testing by DNA in situ hybridization is a pragmatic way of providing HPV testing in clinical practice. A novel RNA in situ hybridization methodology targeting E6 and E7 mRNA has been validated and is likely to be available as an in vitro diagnostic device soon. This review will outline the current concepts around the diagnosis of HPV-associated head and neck SCC and suggest a diagnostic algorithm that can be instituted in most diagnostic cellular pathology laboratories.

**Keywords** head and neck; HPV; immunohistochemistry; in situ hybridization; molecular diagnostics; p16

## Introduction

Human papillomavirus (HPV) associated oropharyngeal squamous cell carcinoma (OPSCC) is a subset of head and neck cancer with specific clinical, histopathological and genomic characteristics.[1] The presence of oncogenic high-risk HPV has shown to be a strong independent prognostic factor for OPSCC, irrespective of lymph node status (see article by Powell and Evans in this issue). Therefore, accurate assessment of HPV is important not only for diagnostic purposes, but may emerge as a predictive biomarker in an era of evolving precision medicine. Epidemiological data suggests that the incidence of HPV-associated OPSCC will exceed cervical cancer as the main malignancy associated with the virus, and consequently there is a need to have an internationally agreed testing strategy that is both reliable and cost-effective.[2]

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## Indications for HPV testing in head and neck cancer

High-risk HPV infection demonstrates a striking site-specificity with the oropharynx, and testing is recommended for those tumours arising in this site, which includes the soft palate, palatine tonsils, base of tongue and posterior pharyngeal wall. HPV testing is also indicated for metastatic squamous cell carcinoma to lymph nodes in the neck from an unknown primary. The Royal College of Pathologists (UK), College of American Pathologists and the National Comprehensive Cancer Network (USA) have all issued guidelines recommending HPV testing in these settings.[3–5]

Using validated algorithms, the presence of high-risk HPV has also been reported in a minority of carcinomas arising from non-oropharyngeal upper aerodigestive tract mucosa, namely the oral cavity, sino-nasal tract, hypopharynx and larynx.[6] More recently, there has been some suggestion that the presence of high-risk HPV in carcinomas at these sites may have similar prognostic implications as those tumours arising within the oropharynx.[7,8] However, these reports are relatively preliminary and routine HPV testing in head and neck squamous cell carcinomas in non-oropharyngeal sites is not currently recommended. Nevertheless it is conceivable that as data accumulates there may be a persuasive argument to screen all head and neck cancers for oncogenic HPV infection.

Furthermore, there have been reports of HPV-associated dysplastic lesions in the oral cavity and there are a growing number of reports showing high-risk HPV in association with non-squamous tumour types arising in the oropharynx,[9] (see article by Bishop in this issue). At present, the biological significance of HPV infection in these scenarios is not clear and therefore routine testing is not currently recommended.

### HPV detection methods

Testing for HPV-associated OPSCC is underpinned by demonstrating biologically relevant HPV infection, where malignant cells should be seen to harbour a high-risk HPV genotype that leads to oncogenesis via the E6 and E7 oncoproteins. The currently accepted reference test or 'gold standard' for HPV is the demonstration of transcriptionally active high-risk HPV E6/E7 mRNA on fresh frozen material by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR).[10] Whilst this test is feasible in the research laboratory setting the assay has not been adopted by clinical laboratories mainly due to the labile nature of RNA extracted from fresh tissue and workflow protocols based around the processing of formalin fixed tissue. Clinical tests should be subject to thorough validation and quality assurance, and should ideally be cost-effective and meet acceptable turnaround times. Here, we provide a summary of the different techniques available for HPV detection, with the aim of outlining the current diagnostic algorithms that could be employed in a routine diagnostic laboratory.

 

The assay of choice used for HPV detection depends primarily on the nature of the diagnostic sample (e.g. fresh; or alcohol- or formalin-fixed), and also on the preservation state and the ubiquity of the target molecule (e.g. DNA, RNA or protein). HPV testing is either aimed at the identification of a group of high-risk HPV genotypes or targeting specific high-risk HPV types, for example, HPV-16, the most common genotype in OPSCC. HPV detection methods use either target or signal amplification. Polymerase chain reaction (PCR) based techniques use target amplification of DNA or cDNA from reversely transcribed RNA (RT-PCR), whilst signal amplification techniques identify targets by chemical amplification of a colourimetric marker. A summary of the different HPV tests is provided in Table 1. The following is an overview of the techniques that have demonstrated greatest utility and shown the most consistent evidence for use in OPSCC within the diagnostic histopathology setting.

### Immunohistochemistry

**p16 immunohistochemistry:** p16 immunohistochemistry (IHC) is widely used in the diagnostic laboratory as a surrogate marker for HPV infection in OPSCC. p16 is a tumour suppressor gene that acts as an inhibitor of the cyclin dependent kinase 4A. The accumulation of p16 protein in OPSCC is due to binding of the HPV oncoprotein E7 to Rb protein, which inhibits its negative feedback on p16 (see article by Powell and Evans in this issue). In contrast, SCC in the head and neck that is not associated with HPV is typically negative for p16. p16 overexpression has a sensitivity approaching 100% for high-risk HPV infection, but its specificity is low at 79–82% due to false positive staining in the absence of HPV infection.[11]

p16 IHC can be carried out using proprietary reagents (p16 clone E6H4, CINtec Histology, Roche mtm laboratories). The antibody is supplied as a 'ready to use' (RTU) product for the Ventana Benchmark autostainer (Ventana Medical Systems Inc) and as a dispensable kit for use on other automated staining platforms and for manual 'bench top' staining methods. A previous OPSCC with p16 overexpression or known cervical intraepithelial neoplasia 3 (CIN3) lesion can be used as a positive control. Normal crypt epithelium shows moderate positivity for p16 and serves as a useful internal control for staining

**An overview of currently available HPV tests**

| Target | Technique | Advantages | Disadvantages |
|---|---|---|---|
| HPV DNA | Southern blotting | • High copy number required for detection | • High quality and quantity DNA from frozen tissue needed<br>• Low sensitivity |
| | In situ hybridization | • High specificity<br>• Suitable for FFPE material<br>• Allows direct visualization of viral DNA co-localizing with nucleus | • Low sensitivity |
| | Consensus PCR and genotyping | • Highly sensitive<br>• Suitable for FFPE material | • Positive result does not distinguish biologically relevant infection from transient infection<br>• High risk of contamination and false positivity |
| | Type-specific PCR | • High sensitivity<br>• Suitable for FFPE | • Similar to consensus PCR |
| | Real time PCR | • High sensitivity and specificity<br>• Suitable for FFPE<br>• Give an accurate estimation of viral load | • Laser capture micro-dissection of tumour cells required |
| HPV RNA | Reverse transcriptase PCR | • Highly sensitive and specific<br>• 'Gold standard' test | • Limited use to fresh frozen tissue |
| | HPV RNA ISH | • High sensitivity and specificity<br>• Demonstrates active transcription of HR HPV | • Limited use at present in research setting |
| HPV proteins | IHC for E6 and E7 | • Allows demonstration of oncogenic proteins | • Suboptimal IHC performance |
| Surrogate protein markers | p16 IHC | • Highly sensitive<br>• Easy to interpret<br>• Suitable for FFPE<br>• Surrogate marker for biologically relevant HPV infection<br>• Prognostic marker<br>• Cost-effective | • Low specificity |
| | pRB IHC | • Suitable for FFPE material | • Low expression difficult to interpret |
| Serum antibodies | HPV protein antibodies | • Minimally invasive | • Low sensitivity and specificity |

**Table 1**