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Formalin fixed paraffin embedded (FFPE) material is amenable to HPV detection by the Xpert® HPV assay

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ABSTRACT

Background: The Xpert® HPV Assay (Cepheid®, Sunnyvale, USA) is a rapid, cartridge-based HPV test validated for use on cervical cytology samples. However, there is an increasing demand for HPV annotation of formalin fixed paraffin embedded (FFPE) material.
Objectives: The aim of this study was to determine the suitability of the Xpert HPV assay for the detection of nucleic acid (NA) derived from FFPE samples.
Study design: A total of 88, 10 μm sections derived from FFPE tissue blocks were assessed, 74 originated from oropharyngeal squamous cell carcinomas (OPSCC) and 14 from a range of other sites. All had previously been tested with a sensitive Luminex® based assay with a component also tested with p16 immunohistochemistry (IHC). NA was extracted from samples using the easyMag® platform and after dilution was added directly to the Xpert cartridge.
Agreement between assays was assessed.
Results: Overall agreement between the assays was 92%; with a Kappa for HR-HPV detection of 0.833 (95% CI 0.725–0.953). In the 50 samples that had been annotated for p16 status overall agreement between the Xpert assay and the p16 IHC was 90%.
Conclusions: These data indicate that FFPE material is amenable to HPV detection by the Xpert assay. To our knowledge, this is the first study to interrogate the use of the Xpert® HPV assay for this application.

1. Background

Human papillomavirus (HPV) infection causes neoplasms of the anogenital tract and the head and neck, with morbidity for the latter largely associated with oropharyngeal squamous cell carcinoma (OPSCC). The incidence of OPSCC has increased over the past 20 years in Europe, the United States of America and Australia [1–3]. In Scotland, HPV infection is associated with approximately 50% of all cases of OPSCC [4,5]. However, prevalence of HPV associated OPSCC varies between countries with data to suggest that it may be higher in North America than in Europe [6].

As a consequence, annotation of OPSCC is helpful for epidemiological purposes. In addition, as HPV positive OPSCC is associated with improved clinical outcomes [7–9]; knowledge of HPV status provides insight into prognosis and may, in future, be used to inform treatment modalities [10]. The best method by which to confirm HPV status in OPSCC is somewhat controversial although it has been proposed that a dual approach of testing for HPV nucleic acid and p16 immunohistochemisrty is optimal [11–13]. p16 is a surrogate marker of deregulated E7 oncoprotein expression (as a result of inactivation of pRB); and there is some evidence that this p16 immunohistochemitry may be more specific than nucleic acid (NA) testing for identification of HPV driven OPSCC [10].

Certainly, there is an increasing demand for HPV testing of formalin fixed paraffin embedded (FFPE) material for research, epidemiological and clinical indications and while a variety of tests are available for the detection and genotyping of HPV which differ in scope and automation [14,15], relatively few commercial assays have been validated for FFPE material [16].

The Cepheid® Xpert® HPV assay is a recently developed assay, is cartridge-based and validated for use on cervical liquid-based

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Table 1
HR-HPV detection by the Luminex assay vs. Xpert assay in the total sample ($n = 88$).

HR-HPV		Luminex		
	+	–	Total	
Xpert HPV	+	35	1	36
	–	6	46	52
	Total	41	47	88

cytology samples. It can detect 14HR-HPV (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) in less than one hour. [17].

Given the demand for HPV annotation of FFPE material for clinical and epidemiological applications, our objective was to evaluate the suitability and performance of this assay for use with FFPE samples.

The amplicon length for the Xpert HPV assay is in the range of 80–150 bp across the 14HPV targets and the Luminex assay generates a PCR product with an expected length of ~150 bp [18].

2. Objective

The objective of the study was to determine the suitability of the HPV assay for the detection of nucleic acid derived from FFPE samples.

3. Study design

3.1. Sample set

A total of 88, 10 μm sections derived from different FFPE tissues were assessed. Seventy four originated from OPSCC and a component of the samples ($n = 50$) had also been tested for p16 and constituted a cohort from the East of Scotland described previously [5]. The timeframe for initial biopsy collection was from 2011–2015. Briefly, immunohistochemistry for p16 was carried out on 3-μm sections using a monoclonal antibody to p16 (CINtecHistology, mtm Laboratories) on a Leica Bond III automated immunostainer. Sections of normal tonsil were used as a positive control. p16 IHC was considered to be positive if there was strong and diffuse nuclear and cytoplasmic staining present in greater than 70% of the malignant cells.

In addition a total of fourteen samples derived from non-OPSCC sites were assessed including eight vulval cancers, one larynx, one cervical lymph node, one conjunctiva, one neck mass, one vocal cord and one metastasic disease in lymph node (unknown primary). Use of material for the present work was approved by NRS Lothian Bioresource (Reference SR361).

Residual sample sections collected at SHPVRL as a result of routine/service work streams were stored at −80 °C for up to 5 months, where total nucleic acid was extracted (NA) using the easyMag (Biomerieux®, Marcy-l'Étoile, France) platform using an existing protocol adapted for FFPE material [16].

All samples had been tested previously using the Diamex® HPV Genotyping Kit, (Heidelberg, Germany) assay based on Luminex® (Austin, USA). This is an in vitro test with hybridization and fluorescence detection for the qualitative detection of 24HPV genotypes including all established High-Risk types [18].

Table 2
HR-HPV detection by the Luminex vs. Xpert assay in the OPSCC set ($n = 74$).

OPSCC		Luminex		
	+	–	Total	
Xpert HPV	+	30	1	31
	–	6	37	43
	Total	36	38	74

3.1.1. Controls

The Xpert HPV assay includes a human gene as reference (hydroxymethylbilane synthase [HMBS]) and an internal probe check control (PCC) in each cartridge.

A minimum of one positive and one negative control were included on each day of testing. Positive control used was SiHa cell line HPV 16 positive, agglutinated and embedded into a paraffin block from which 10 μm sections were cut. Blank paraffin sections without any tissue or cell line material (10 μm) were used as negative controls.

3.1.2. Pre-extraction treatment

To be able to extract the NA from the FFPE material, samples were initially deparaffinised. In brief, paraffin was removed from each sample by adding 320 μL of deparaffinization solution (Qiagen®, Venlo, Netherlands), followed by a brief pulse centrifugation and 3 min incubation in a waterbath at 56 °C.

A 10 μL volume of Tween 20 (Sigma®, St. Louis, USA) and 180 μL of DNAse/RNAse free water (Sigma®) and 20 μL of Proteinase K (Qiagen®) were added directly to each sample emulsion, followed by a 1 min centrifugation at 7378 × *g* and overnight waterbath incubation at 56 °C.

3.1.3. NA extraction

A 200 μL volume of the lysed sample emulsion was added to 2 mL of Lysis Buffer (Biomerieux®). The mixture was then added to the easyMAG (Biomerieux®) vessel and 100 μL of diluted silica (600 μL of silica (Biomerieux®) + 600 μL of Nuclease Free Water (Omega Bio-tek Inc., Norcross, USA®).

NA was extracted on the EasyMAG machine using the "Generic 2.0.1" program. Elution was performed in 110 μL volume of NucliSens Extraction buffer 3 (Biomerieux®). NA sample yield was measured using a Nanodrop® spectrophotometer (Thermo Fisher Scientific, Waltham, USA).

3.1.4. Cepheid Xpert® HPV assay

A 110 μL volume of the NA extract was added to 890 μL of DNAse/RNAse free water (Sigma®). A 1 mL volume was subsequently transferred into the sample chamber of the Xpert HPV cartridge according to the manufacturer's protocol [6] and inserted in the GenXpert® module for HPV detection.

Results were obtained within 60 min for the presence/absence of HPV genotypes. A positive result was reported as either 16, 18–45, 31–35–33–52–58, 51–59, 39–68, 56, 66 together with a Ct value.

3.1.5. Analysis

The Cohen's Kappa test was used to assess agreement between the Luminex and the Xpert test for the total sample set, and also for the OPSCC samples separately. For the purpose of the analysis invalid results obtained on both assays were classified as "negative".

The McNemar's test was performed to assess if the distribution of discordant results was significant. Discrepant analysis was performed descriptively. Agreement of the Xpert assay with p16 and McNemar's test (on the subset of samples where this had been performed) was also assessed.

4. Results

4.1. NA yields

NA quality and quantity were analysed with the Nanodrop® obtaining yields ranging from 7 to 227 ng/μL, mean 42.15. The ratio