Evaluation of a new HPV detection technology ‘the INFINITI® HPV genotyping assay’ for use in HPV associated cancers and pre-cancers

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Introduction
HPV is a sexually transmitted virus associated with the development of genital warts, pre-cancers lesions, cervical cancer and head and neck cancer. HPV is a self-limiting infection that eventually resolves. However, in 10% of cases, the virus persists, producing a transforming infection.

The HPV oncoproteins E6 and E7 are responsible for producing the transforming phenotype. HPV types 16/18 are human carcinogens and are associated with the development of 97% of cervical cancers and up to 72% of head and neck cancers.²,³

In Ireland alone, approximately 170 women and 438 men are diagnosed with head and neck cancer each year. Risk factors for the development of head and neck cancers include smoking and alcohol intake.

Objective
• Evaluate the INFINITI® HPV-HR QUAD assay for the detection of HPV in FFPE head and neck tumours.
• To validate the assay for the detection of HPV in cervical PreservCyt® samples.
• Test the reproducibility of the assay by performing dilution series using SiHa cell DNA.

This technology is only recommended for the detection of HPV in cervical samples and is our hope that the outcome of this project could result in the modification of this assay for use on FFPE head and neck tumour samples.

Materials & Methods
• Cervical cell lines; SiHa, CaSk, C33a and HeLa were made into cell blocks and formalin-fixed and paraffin embedded to use as cervical controls.
• Cervical PreservCyt® samples (n=20) were used as part of the validation study of the INFINITI®, and were previously tested on the Cobas® 4800.
• Head and neck tumour samples (n=102) from the CERVIVA biobank were used for HPV DNA analysis on the INFINITI®.
• The head and neck samples were previously immunostained for p16, SPF-10 PCR tested and HPV genotyped by the INNO-LiPA®.

The extracted DNA was amplified by PCR using the GeneAmp® PCR system 9700, each cycle lasting 2 hours.

The PCR product was then loaded onto the INFINITI® analyser in a 48 well plate, and the HPV-HR QUAD assay was selected. Each run lasted up to 5.5 hours.

Results
Detection of HPV by the INFINITI® in a cervical cohort:
Among the (n=20) cervical samples chosen for the study, 3 were HPV negative and 17 were HPV positive. The INFINITI® detected all samples that were HPV positive and generated them according to the Cobas® 4800. The overall agreement between the INFINITI® and the Cobas® 4800 HPV test was 100% (95% C.I. 1.00;1.000).

Detection of HPV by the INFINITI® in a head and neck cancer cohort:

Figure 1: The concordance between the INFINITI® and the INNO-LiPA® for the detection of HPV in FFPE head and neck cancer samples. From a cohort of (n=102) head and neck cancer samples, the INNO-LiPA® detected 66 HPV+ cases and 50 HPV- cases. The overall agreement between the INNO-LiPA® and the INFINITI® was 74.51% (F-cox, P=1.082; 7.802).

Figure 2: The relationship between p16 positive head and neck tumours and disease survival. Patients with HPV+ (p16+)/head and neck tumours have an increased survival rate compared to HPV- (p16-) tumours.
P16 positivity and survival rate

Figure 3: The % of HPV+ tumours within groups based on weekly units of alcohol consumed. There is no correlation between alcohol and HPV positivity within the selected cohort. 50% (25/50) of Patients consuming <10 units/week had HPV+ tumours. While 67% (41/62) of patients consuming >10 units/week had HPV+ tumours.

Figure 4: Smoking status of head and neck cancer patients. A total of 68% of current smokers and 48.6% of ex-smokers had HPV+ positive tumours while non-smokers showed a slight decrease in HPV+ tumours at 61%.

Figure 5: The incidence of head and neck tumour subtypes within a cohort (n=102). The most commonly occurring head and neck tumour subtype was squamous cell carcinoma of the oral cavity (35/102 (34.3%)) followed by oropharynx (23/102 (22.83%)) and hypopharynx (16/102 (15.73%)). The most frequently HPV+ affected site was the oral cavity (47/102 (46.10%) patients, 34/47 (77%) of which were HPV+ positive.

Conclusion
The assay is only recommended for the detection of HPV in cervical specimens and our results would support this. However there are several factors which need to be taken into consideration, such as; the fixation process causes cross-linking of nucleic acids which can contribute to the poor quality DNA extracted from FFPE tissue, paraffin wax can also interfere with DNA extraction. To resolve these challenges we would suggest including a second xylene step during DNA extraction to ensure there is no paraffin wax remaining which could interfere with DNA quality. A novel method of fixation known as HOPE could replace formalin fixation. HOPE fixation does not alter DNA structure, it maintains proteins and antigenic structure while also maintaining good tissue morphology. The cut-off value of the INFINITI® HPV-HR QUAD assay is 2,000 copies of HPV DNA, which is considerably higher than that of the Cobas® 4800 (300 HPV copies) and the INNO-LiPA® (20-70 copies). The high cut off point of the INFINITI® HPV-HR QUAD assay may explain the high rate of false negatives. It is our recommendation that further studies are performed using a larger cohort, while also addressing the issues of fixation and the cut off point of the assay.