Technical External Quality Assessment Schemes for p16 and High-Risk Human Papilloma Virus Detection in Head and Neck Squamous Cell Carcinoma: Report of Year-One Results

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Background:

Head and neck squamous cell carcinoma (HNSCC) contributes 6.5% of the annual cancer cases seen worldwide.

Guidelines published in the USA and Europe call for the universal testing of all newly diagnosed oropharyngeal squamous cell carcinomas (OPSCCs) for p16 over-expression as a surrogate for transcriptionally-active high risk Human Papilloma Virus (HPV), with which it has been shown to be very highly correlated (almost 100% sensitivity and 90% specificity).

This is as an aid to the identification of HPV-positive OPSCC, which has distinct clinicopathological features and a favourable prognosis.

Aims:

We report here the data from a newly established external quality assessment (EQA) programme for p16 and HPV.

Methods:

Sections from formalin fixed paraffin embedded (FFPE) specimens were prepared onto glass microscope slides and circulated to participating laboratories.
The following sample types were used: HNSCC showing differing levels of p16 and HPV expression; HNSCC tumours in which neither marker was demonstrable; tonsil showing reactive changes
Stained slides were returned for central assessment.

Results:

p16: Overall there were 143 participations, of which 140 (97.9%) were assessed as acceptable (inter-Run range (IRR) = 94.3-100.0%).
In the acceptable category 126 (88.1%) were further categorized as being of good/excellent quality (IRR = 83.3-90.0%).
The primary antibody most used was the CINtec E6H4 antibody (Ventana Medical Systems), used by 78 (81.3%) of the 96 laboratories that reported their method. All 78 submissions that used this antibody (100.0%) demonstrated acceptable stain quality. See Charts 1 & 2.

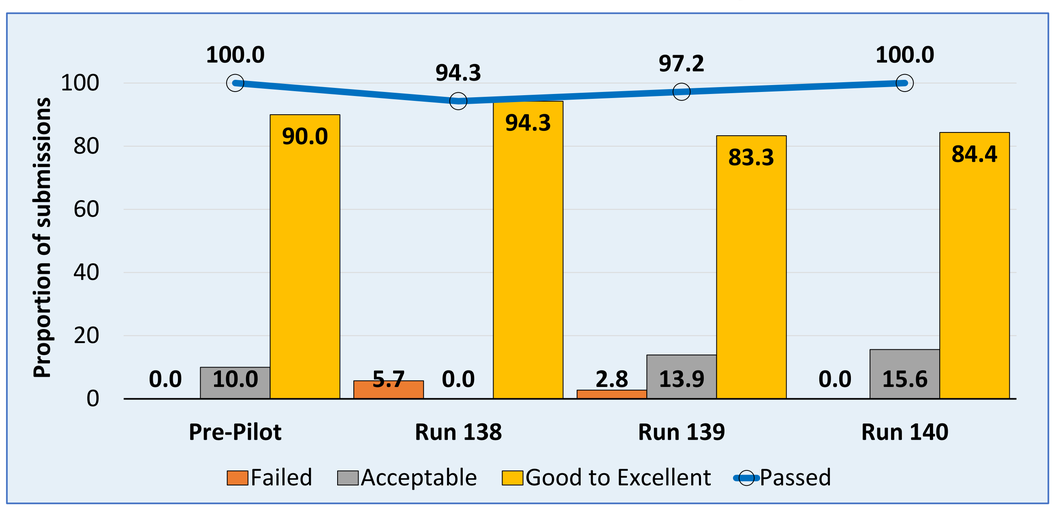

Chart 1. Showing proportions of submissions categorised according to stain quality at each Run (columns), and the overall proportion passing the assessment (line).

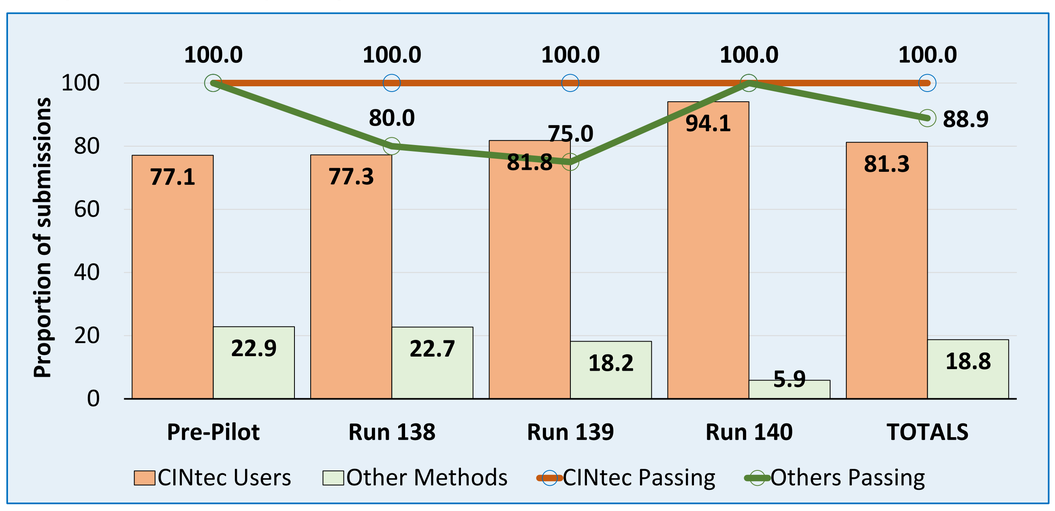

Chart 2. Showing proportions of submissions categorised according to antibody used at each Run (columns), and the overall proportions passing the assessment (lines).

HPV: In total there were 43 submissions, of which 42 (85.7%) were assessed as acceptable (IRR=75.0-92.3%). 31 (63.3%) were of good/excellent quality (IRR=46.2-84.6%). The probe set most used was the HPV III Family 16 Probe (VMS), used by 34 (82.9%) of the 41 laboratories reporting their method. 29 (85.7%) produced submissions of acceptable stain quality. See Charts 3 & 4.

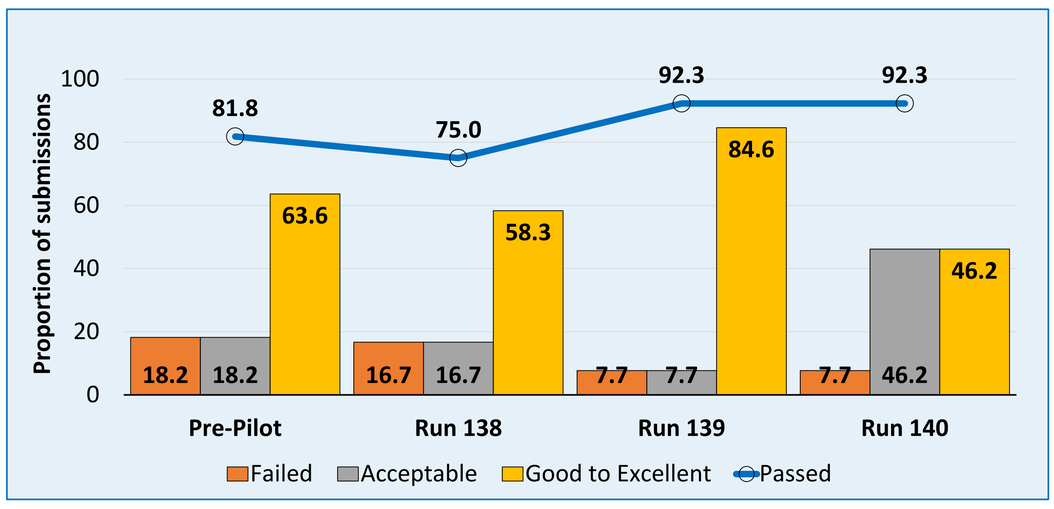

Chart 3. Showing proportions of submissions categorised according to stain quality at each Run (columns), and the overall proportion passing the assessment (line).

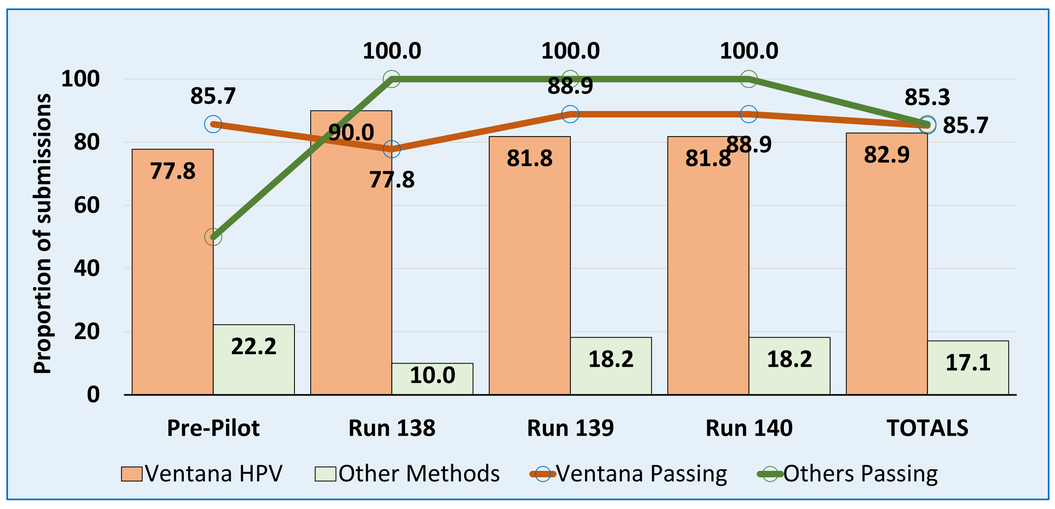

Chart 4. Showing proportions of submissions categorised according to antibody used at each Run (columns), and the overall proportions passing the assessment (lines).

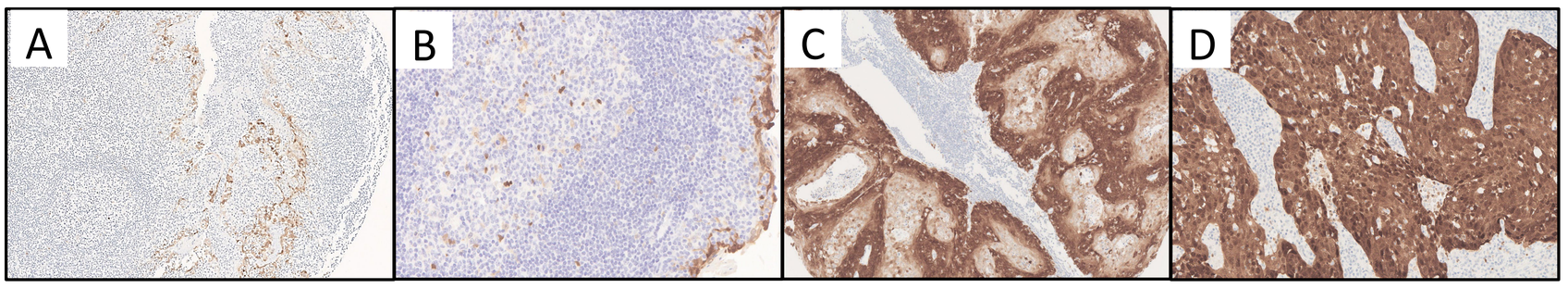

Figure 1: UK NEQAS distributed samples showing the expected level of P16:
(A) Tonsil (x10), (B) Tonsil (x20),
(C) Positive HNSCC, (D) Positive HNSCC

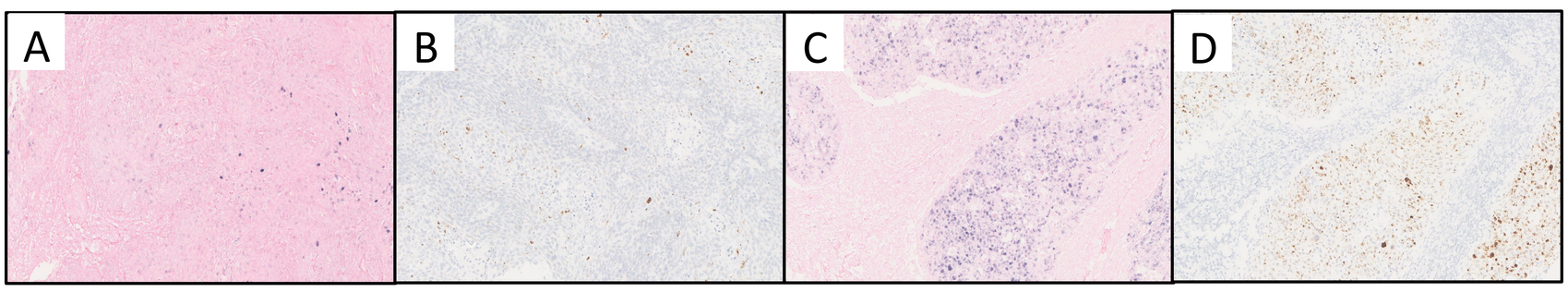

Figure 2: UK NEQAS distributed samples, expected level of HPV:
(A) Low expressing positive HNSCC stained with Roche HPV probes (x20), (B) Low expressing positive HNSCC stained with RNAScope (x20), (C) High expressing positive HNSCC stained with Roche HPV probes (x20), (D) High expressing positive HNSCC stained with RNAScope (x20)

Conclusions

In the H&N SCC setting, p16 IHC is done exceptionally well in most laboratories submitting material to the EQA. Similarly, detection of HPV in the same type of material is also performed well, albeit with slightly more variability. The most commonly used primary markers in each of the EQAs both produced superior results when compared to the aggregate for all the alternatives.