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Clinical sensitivity of HPV assays for the detection of high grade cervical disease in cervical samples treated with glacial acetic acid

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ABSTRACT

Background: Lysis of bloody liquid based cytology (LBC) specimens with glacial acetic acid (GAA) is performed to aid cytological interpretation. However, the influence of GAA treatment on HPV detection is not fully understood and in studies designed to assess this, few cases of high-grade disease have been included.

Objectives: To assess the sensitivity of HPV molecular tests for the detection of high grade cervical disease in GAA treated samples

Study design: A total of 207 specimens associated with high grade dyskaryosis and treated with GAA were collated prospectively. Overall 140 specimens had underlying CIN2+, including 88 CIN3. All specimens were tested with the Abbott RealTime High Risk HPV test (*rt*HPV) and the Qiagen Hybrid Capture 2High Risk HPV DNA test (HC2). Specimens associated with a CIN2+ that were negative by either assay were genotyped.

Results: The sensitivity of *rt*HPV for CIN2+ and CIN3+ was 92.8% (87.2, 96.5) and 94.3% (87.2, 98.1) respectively. Sensitivity of the HC2 for CIN2+ and CIN3+ was 97.2% (92.8, 99.2) and 96.6% (90.3, 99.2) respectively. The sensitivity of both assays in GAA treated specimens was thus consistent with the level required for clinical application. HPV negative, CIN2+ specimens were generally attributable to HPV types outside the explicit analytical range of the assays.

Conclusions: The data indicate that GAA treatment has little impact on the detection of CIN2+ by HPV testing in LBC specimens.

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1. Background

Cervical LBC specimens with high red-blood cell content are often treated with glacial acetic acid (GAA) to lyse erthythrocytes as an aid to cytological interpretation [1]. While the proportion of specimens treated with GAA is difficult to quantify globally due to a lack of consistent reporting, a recent Scotland-wide survey of the cytology laboratories serving the national cervical screening programme showed the proportion of lysed specimens to be 4.8% overall with a range of 1.6–10% (data not shown).

As an increasing component of cervical screening and associated disease management relies on HPV testing as a reflex or co-test with cytology [2], it is important to assess whether GAA has an impact on molecular HPV assays and if this impact is differentially exerted across the different assay chemistries and platforms. Of the small number of studies where the impact of GAA has been assessed, the evidence would indicate that the COBAS 4800HPV Assay (Roche Molecular Systems, Pleasanton, CA, USA) and APTIMA HPV Test (Gen-Probe Inc., San Diego, CA) are unaffected by GAA treatment – as opposed to the Cervista Test (Hologic, Inc., Bedford, MA) where it has a deleterious influence [3–6]. Furthermore, in an earlier split-specimen study of untreated vs treated specimens undertaken in our laboratory, while the impact of GAA on HPV detection was insignificant at the qualitative level, GAA treatment was associated

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with lower read-outs according to the semi-quantitative measures of the assays chosen: HC2 and *rt*HPV [7].

An important limitation of the studies described above is that the assessment of GAA influence on HPV testing has largely been determined at the qualitative level in relatively small analytical studies with, crucially, a lack of knowledge of the underlying pathology or, if pathology was known, only a few cases of high-grade disease. For example, in Munson et al. no pathology (cytology or histology) status was provided, whereas in the studies of McMenamin et al. [3,6] and Moore et al. [7] while cytology was reported, only 17/121 (14.0%) and 4/150 (2.6%) cases respectively were associated with high grade cytology—and no histological data was presented. The present article builds on existing data by focussing on the assessment of a key clinical-performance measure of HPV assays (sensitivity for the detection of CIN2+) in GAA treated samples, rather than focussing solely on analytical performance.

2. Objectives

To compliment and consolidate existing data, the present study was designed to assess the clinical sensitivity of two clinically validated HPV assays for histologically confirmed, disease in cervical samples treated with GAA.

2.1. Study design

2.1.1. Specimen collection and annotation

The two largest cytology laboratories in Scotland which serve the Scottish Cervical Screening Programme (SCSP); Greater Glasgow and Clyde (GGC) and Lothian participated in the study. Both laboratories prospectively collated LBC specimens that (1) indicated “routine” GAA treatment, (2) were graded as high-grade dyskaryosis according to the British Society for Clinical Cytology (BSCC) criteria [8]. Specimens with underlying high-grade dyskaryosis were selected to enrich for CIN or worse (CIN2+) lesions and a total of 214 specimens (ThinPrep® PreservCyt®) were collated over a 12 month period. In Scotland a result of high-grade dyskaryosis triggers a referral to colposcopy. Histology results associated with these cases were captured via the Scottish Cytology Call Recall System (SCCRS): an integrated IT system that serves the Scottish Cervical Screening Programme (SCSP) and contains cytology, colposcopy and histology information. Of the 214 specimens collected, 7 were excluded from analysis owing to insufficient volume for HPV testing, leaving a total of 207 for assessment. The underlying pathology of the 207 evaluable specimens was as follows −5 (cases of) histology negative, 34 CIN 1 lesions and 140 CIN2+ lesions, which incorporated 78 CIN3 lesions and 10 carcinomas. Histology was not available for 19 cases and no biopsy was taken in a further 9.

Permission to deliver the work as a service development project was provided by the South East Scotland Research Ethics Service (NR/1101AB4).

2.1.2. GAA treatment protocol

Both cytology laboratories, Greater Glasgow and Clyde (GGC) and Lothian use the Hologic protocol for the processing of ThinPrep Papanicolaou specimens (Hologic, Malborough, MA, USA) with slight variations described previously [7]

2.1.3. HR-HPV detection

The 207 samples with sufficient volume were tested by the HC2 test and *rt*HPV. To mimic a routine/service testing environment samples were tested only once by each assay. Testing was according to manufacturers’ instructions—briefly, the *rt*HPV is a target amplification assay designed to detect 14 high-risk types, with identification of HPV 16, HPV 18 and “Other” high-risk HPV types, whereas the HC2 test is a signal amplification assay which detects 13 high-risk types in aggregate. These assays were those used in the split-sample study described earlier [7].

Table 1
Agreement between HC2 and *rt*HPV in the overall specimen set (3a), the 140 specimens associated with CIN2+ (3b) and the 88 specimens associated with CIN3+ (3c).

3a	HC2 result		
*rt*HPV result	Negative	Positive	Total
Positive	2	189	191
Negative	8	8	16
Total	10	197	207

3b	HC2 result		
*rt*HPV result	Negative	Positive	Total
Positive	1	129	130
Negative	3	7	10
Total	4	136	140

3c	HC2 result		
rtHPV result	Negative	Positive	Total
Positive	1	82	83
Negative	2	3	5
Total	3	85	88

2.1.4. HPV genotyping

Specimens associated with CIN2+ which tested HPV negative by either or both the HC2 or *rt*HPV tests were also subject to HPV genotyping using the Linear Array HPV Genotyping Test – LA, (Roche Molecular Systems, CA, USA) and the Optiplex HPV Genotyping Assay (Diamex, Heidelberg, Germany). Both genotyping assays were performed according to manufacturers’ instructions. Briefly, the LA assay involves reverse line blot hybridisation of PCR products and can delineate 37HPV types (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 58, 59, 61, 62, 64, 66, 67, 68, 69, 70, 71, 72, 73 (MM9), 81, 82 (MM4), 83 (MM7), 84 (MM8), IS39 and CP6108) whereas the Optiplex assay uses luminex fluorescent bead array (FBA) technology and detects 24 genotypes: 6,11,16,18, 26, 31, 33, 35, 39, 42, 43, 44, 45, 51, 52, 53, 56, 58, 59, 66, 68, 70, 73, 82.

2.1.5. Analysis

The design of the study precluded the capture of a pre-treatment aliquot as cytology status could only be confirmed in real-time after GAA treatment. However, for an HPV assay to perform according to accepted performance standards, sensitivity for CIN3 or worse (CIN3 +) of at least 90% (+/−) 3% should be achieved [9]. Sensitivity comparisons of the HC2 and *rt*HPV tests were performed using Fisher’s exact test to assess the non-random associations between two variables. HPV genotype status of HPV negative CIN2+ specimens was analysed descriptively given the small number of cases (n = 11).

3. Results

3.1. HPV positivity and agreement between HC2 and rtHPV

As expected, HPV prevalence was high in this study population with 197/207 (95.2%) and 191/207 (92.3%) testing positive by the HC2 and *rt*HPV assays respectively. Agreement between the assays was high (Table 1), being 95.2% overall (n = 207), 94.3% in the 140 cases associated with CIN2+ and 95.4% in the 88 cases associated with CIN3+.

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