External quality assessment for molecular detection of human papillomaviruses

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Abstract

External quality assessment (EQA) of the cytological diagnosis of cervical cancer is well established in the UK. The introduction of direct high risk (HR) human papillomavirus (HPV) testing, into national cervical screening programmes, would necessitate an EQA scheme. An EQA scheme for HPV DNA testing was developed by the Royal Infirmary of Edinburgh in 2005 for the NHS Cervical Screening Committee HPV/LBC Pilot study with a primary objective of including the EQA for HPV within United Kingdom National External Quality Assurance Service (UK-NEQAS) for Microbiology provision. The suitability of specimens for this UK NEQAS scheme was determined by distributing three Molecular detection of HPV pilot distributions and assessing the methods used to analyse the data and participants’ performance.

To assess the suitability of using liquid based cytology (LBC) samples for EQA of molecular methods and to review the methods used by participants to detect the presence of high risk (HR) human papillomavirus (HPV) genotypes.

Methods

• EQA specimens were prepared using residual LBC samples from the routine cervical screening population in Edinburgh and tested twice using HR-HPV Hybrid Capture II (hc-2) DNA test (Digene, now Qiagen) in different assay runs.
• Samples were diluted or pooled/diluted to produce sufficiently large volumes of EQA specimens, and re-tested to ensure reproducible pre-distribution results.
• Pre-distribution tests were carried out separately on all specimens by the Scottish HPV Reference Laboratory and by the Virus Reference Laboratory, HPA Centre for Infections, London; hc-2 assay, HPV Amplior (Roche Diagnostics), HPV Linear Array (LA) (Roche Diagnostics) and an in-house real-time multiplex PCR.
• Three pilot distributions were dispatched between January 2008 and January 2009 with a request to report on the detection of HPV by molecular methods.
• Of the twelve specimens dispatched eight were positive for high risk HPV genotypes, four were negative for high risk HPV genotypes (table 1).

Results

Table 1. Description of specimens

<table>
<thead>
<tr>
<th>Distribution</th>
<th>Specimen</th>
<th>Comments on bulk material</th>
<th>HR HPV genotypes</th>
<th>LR HPV genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>2088</td>
<td>8772</td>
<td>Pooled</td>
<td>31, 53</td>
<td>54</td>
</tr>
<tr>
<td>8773</td>
<td>Pooled</td>
<td>negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8774</td>
<td>Single</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8775</td>
<td>Preamplified with MRC-5 cells [0.5 x 1 x 10^6 cells mL^-1]</td>
<td>negative</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 1. Participant performance for each of the 12 specimens distributed from January 2008 to January 2009. (Red: high risk HPV positive; Blue: high risk HPV negative).

Figure 2. Assays used by participants during the pilot phase.

Figure 3. Performance of assays used during the pilot phase.

Figure 4. A single sample positive for HPV high risk genotypes 16, 18 and 59. These results are representative of the types of results seen with other samples containing multiple HR HPV genotypes. The viral load of individual genotypes was not assessed.

Conclusions

• The UK NEQAS scheme for Molecular Detection of HPV provides a homogeneous and characterised specimen that is relevant to the clinical needs of the participants.
• Genotyping results reported by participants still showed variation (figure 4), even between those using the same method, demonstrating the need for an EQA scheme.
• Up to seven different extraction methods were reported to be used for one genotyping method, which most probably results in the variation of genotypes reported by participants using the same genotyping method.
• For example, in the most recent distribution three different extraction methods were used in conjunction with Roche LA: Qiagen, Roche and NucliSENS easyMAG.
• Robust EQA of HPV molecular screening programmes will be essential for monitoring the impact of the HPV vaccine.

Acknowledgements

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References