EXTERNAL QUALITY ASSESSMENT FOR THE DETECTION OF HUMAN PAPILLOMAVIRUSES

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Abstract

The use of human papillomavirus (HPV) testing in gynaecologic practice has extended, leading to more laboratories routinely running such assays and the need for an external quality assessment scheme. The United Kingdom National External Quality Assessment Service (UK NEQAS) introduced a scheme for the detection of high risk (HR) HPV genotypes in 2009. The scheme was initially developed by the Royal Infirmary of Edinburgh in 2005 for the NHS Cervical Screening Committee HPV/LBC Pilot study[1]. Earlier pilot distributions have demonstrated the suitability of the EQA specimens which provide homogenous, characterised and clinically relevant samples[2].

Objective

To review the methods used by participants to detect the presence of high risk (HR) HPV genotypes and to analyse participant performances across 36 specimens distributed.

Methods

- EQA specimens were prepared by the Royal Infirmary of Edinburgh using residual liquid-based cytology samples from the routine cervical screening population and tested twice using HR-HPV Hybrid Capture II DNA test (hc-2, Qiagen) in different assay runs.
- Residual samples were diluted or pooled to produce sufficient volume of EQA specimens.
- Pre-distribution tests were carried out on all specimens by the Scottish HPV Reference Laboratory, Edinburgh and by the Virus Reference laboratory (VRD) HPA, London: hc-2 (Qiagen), HPV Amplicor (Roche diagnostics) or INNO-LiPA HPV Genotyping Extra (Innogenetics), HPV Linear array (Roche diagnoctics) and an in-house real-time multiplex PCR.
- Nine distributions, including three pilot distributions, each consisting of four liquid-based cytology specimens were dispatched with a request to report on the presence of HR HPV.

Participation in the scheme

Participation increased progressively since the scheme was introduced in April 2009 with currently 65 participants (figure 1B) from 15 different countries (figure 1A).

FIG 1A

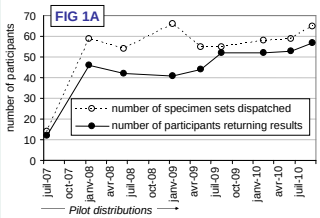
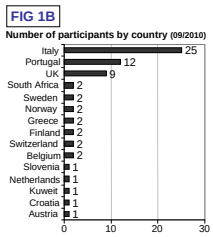


FIG 1B



Methods used by participants

- The most popular assay used to detect HR genotypes was the Hybrid Capture-2 (Qiagen) (Figure 2A)
- In a recent distribution, participants reported the use of 9 different extraction methods. The most commonly used were Qiagen assays, NucliSENS EasyMag (bioMerieux) and MagNApure (Roche).
- Innogenetics INNO-LiPA and Roche Linear Array were the main assays used to determine the genotype (Figure 2B).

FIG 2A

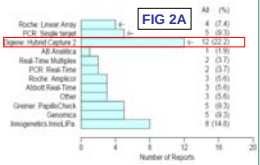
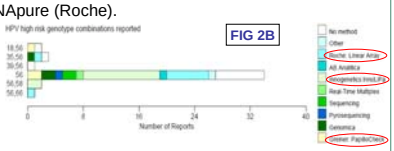


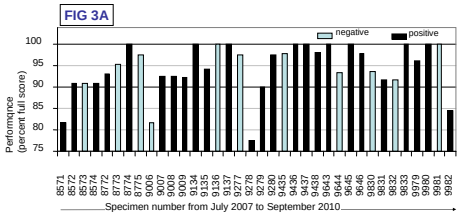
FIG 2B



Performance analysis

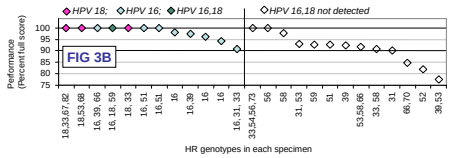
- Overall performance was very good with 94.5% [77.5-100] of participants reporting correctly on the presence of HR HPV genotypes in the 25 positive specimens and 94.6% [81.6-100] reporting correctly on the absence of HR HPV genotypes in the 11 negative specimens Performance below 90% was observed for 4 specimens (Figure 3A).

FIG 3A



- A performance of 81.6% was observed for one negative specimen (#9006). This was most likely due to the presence of low risk genotypes (61, 70 & 81) in the specimen. Performance over 90% was obtained when negative specimens did not were contain low risk genotypes.
- Performances of 81.8%, 77.5% and 84.6% were observed for 3 positive specimens. Specimen 8571 and specimen 8278 were prepared from single positive sample containing HPV HR 52 and HPV HR 39 & 53, respectively. Specimen 8278 was a pooled specimen containing HPV HR 66 & 70. Performance over 90% was obtained when positive specimens contained genotype 16 and/or 18. 100% performance was more frequent in specimens containing genotype 16 and/or 18 (Figure 3B).

FIG 3B



Genotyping

- Although genotype results are not scored, participants can report on the genotypes detected by their assay. The different HPV HR genotype combinations are presented on the reports (Figure 4).

FIG 4

- Intended HR genotype results are determined by the consensus of the results obtained with Roche Linear Array, an in-house multiplex PCR and recently with Innogenetics INNO-LiPA assay.
- Participants report frequently a wider range of HR genotypes and can detect up to 13 extra genotypes alone or in combination (Table 1)

Table 1

Intended	Extra-genotypes detected
52	
33, 58	
39,53	
16,39	
16, 31, 33	39
59	51
31	52,58
16	31,35
31, 53	18,56,33
51	39,68,82
16, 51	33,53,56
33,54,56,73	31,52,66,51
39	16,52,73,59
18, 33	16,39,52,58
16	18,39,44,55
53,58,66	31,33,40,68
16, 18, 59	31,67,51,52,53
18,53,68	33,73,39,45,52
56	18,35,39,58,66
18,33,67,82	16,31,73,39,68,35
16	31,52,56,58,59
58	16,31,33,52,69,71
66,70	11,45,56,60,68,73,82
16, 39, 66	18,31,33,35,45,51,52,56,58,59,68
16,51	11,18,31,33,35,39,45,52,56,58,59,68,82

Conclusion

UK NEQAS has successfully launched the molecular detection of HPV scheme with an overall good performance.

Performance below 90% was observed in 4 specimens out of 36. Results indicated that i) the presence of low risk genotypes in a negative specimen can lead to false positive results and that ii) positive specimens that do not include genotypes 16,18 were less likely to be detected.

Genotype reporting was variable. Although intended high risk genotypes were picked up by the majority of participants, additional high risk genotypes were also reported. Variability in genotype detection can be due the use of detection assays with sensitivity that varies with genotypes and/or using different extraction methods.

In the era of post vaccine implementation, there is a need for data showing HPV vaccine efficacy and to reassess the epidemiology of HPV genotypes. Results of the molecular detection of HPV scheme suggested that current assays may not be as reliable in detecting and identifying high risk genotypes other than 16 and 18.

References

1. Cubie H.A. *et al.* 2005. The development of a quality assurance programme for HPV testing within the UK NHS cervical screening LBC/HPV studies. J Clin. Virol, 33(4):287-292

2. Fagan E.J. *et al.* 2010 External quality assessment for molecular detection of human papillomaviruses. J. Clin. Virol., 48(4):251-4