Detection of HR HPV genotypes in abnormal cervical smears

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PapType, a Molecular HPV Detection System, is High-Throughput, Semi-Automated, and Objective

Background

Cervical cancer is the second most common cancer worldwide and accounts for approximately 1000 deaths annually in the UK. This cancer is prevented by routine cytological screening (Pap Smears) in women aged 25 – 64, but the sensitivity of this screening method to detect high grade cervical intraepithelial neoplasia (CIN2+) and cervical cancer (CA) is highly variable (40-75%) due to the subjective interpretation involved. Currently, up to 16 High Risk Human Papillomavirus genotypes (HR HPV) (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 73 and 82) are considered to be associated with cervical precancer and cancer but some genotypes are known to be more oncogenic than others. For this reason, ***there is a great deal of interest in identifying the specific genotypes present (Szarewski et al. 2012*).*** Here we report our evaluation of a novel method (GENERA Biosystems) for genotyping HR HPV, in both manual (PapType) and semi-automated (PapTypeSP) formats and the predictive value of specific HR HPV genotypes for CIN2+.

Study population:
A total of 1099 women referred to the colposcopy clinics at the Hammersmith and St. Mary's Hospitals in London due to abnormal screening smears (fig.1) were included in this study. CIN2+ determinations were made by histology following colposcopy (fig. 2)

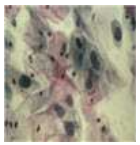


Fig.1. Pap smear showing Dyskaryosis

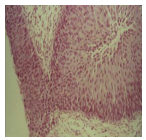


Fig.2. Histology of colposcopic biopsy showing CIN2+.

*Szarewski et al. (2012) J Clin Microbiol 50(6) 1867-1873.

Methods

Sample Prep: DNA was extracted from liquid cytology samples (ThinPrep / Hologic) using Abbott M2000 automated DNA purification system and reagents. Equal amounts of DNA were then used in either PapType or PapTypeSP protocols.

PapType and PapTypeSP: Both versions start with PCR with a red-labelled reverse primer and a set of HPV type-specifc primers which are immobilized on specific beads (fig.3). In the Solid Phase version, PapTypeSP, these beads are included in the PCR and function as forward primers, while in the original PapType the forward primers are in the liquid phase only, requiring steps to make the PCR product single stranded, followed by hybridization. In the PapTypeSP assay, the PCR products are "grown" from the immobilized primers during PCR, reducing handling steps and increasing assay speed, relative to PapType.

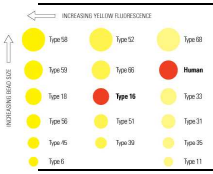
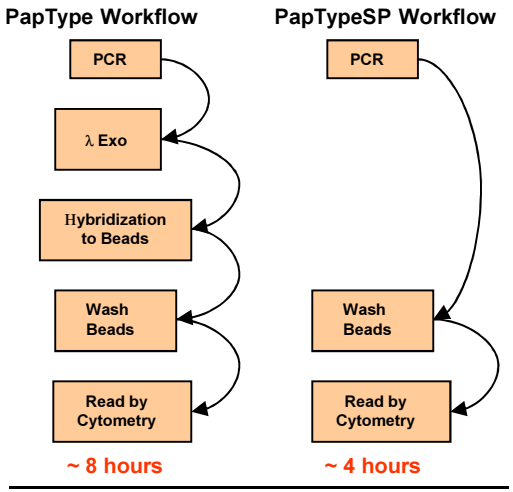


Fig. 3. AmpaSand PapType and PapTypeSP Beadset. HPV type-specific primers are immobilized on beads ranging from 3.0 – 5.6 microns and yellow intensity of 0, half, or full to create a 6 sizes x 3 color intensities (18 cell) matrix. The diagram shows a positive for type 16 + internal control.



Results and Conclusions

The results obtained with PapType and PapTypeSP assays are consistent (k index for HR HPV detection = 0.86). Their performance in detecting CIN2+ is comparable, with Positive Predictive Value (**PPV)** and **Specificity** slightly higher for PapTypeSP than the original PapType.

Genotype	Detection of CIN2+					
	Sensitivity		Specificity		PPV	
	PapType	PapTypeSP	PapType	PapTypeSP	PapType	PapTypeSP
HR HPV	94.6	94.4	22.4	25.3	37.7	38.1
Type 16	54.8	54.4	80.5	82.3	58.3	59.7
Type 18	10.7	9.6	92.1	92.7	40.4	39.1
Type 31	15.8	13.0	91.9	93.0	49.1	47.4
Type 33	16.4	13.6	92.7	93.5	52.7	50.5
Type 35	4.5	4.8	95.1	95.6	31.4	34.7
Type 39	5.6	4.2	91.9	93.9	25.6	25.4
Type 45	5.1	4.2	94.5	95.7	31.6	32.6
Type 51	11.6	10.7	89.6	90.9	35.7	36.5
Type 52	12.1	9.6	91.3	93.0	41.0	40.0
Type 56	2.8	2.5	91.6	92.8	14.3	14.8
Type 58	5.6	5.6	94.5	95.5	33.9	37.7
Type 59	3.1	2.8	96.8	95.5	32.4	23.3
Type 66	3.7	3.4	91.6	90.0	17.8	14.1
Type 68	4.0	4.2	95.4	96.3	29.8	35.7

If one bases addition of a new genotype to a consensus cocktail on the positive predictive value for adding that genotype to those already included, the order after HPV16 would be HPV33, 31, 18, 52, 51, 58, 35, 68, 45, 59, 39, 66 and 56. The detection of the first 6 genotypes would provide a cumulative CIN2+ detection rate comparable to cytology.