Validation of ThermoFisher's Papspin for human papillomavirus detection in cervicovaginal specimens using PCR with GP5+/GP6+ primers and the Hybrid Capture II assay

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Abstract

The present study aimed to validate ThermoFisher's (Thermo Fisher Scientific, Runcorn, Cheshire, UK) Papspin (PS) for human papillomavirus (HPV) testing by in-house PCR and by the Hybrid Capture II (HC2) assay and to compare the results with those obtained using Specimen Transport Medium (STM) (Digene Diagnostics, Gaithersburg, MD, USA). Forty-five patients underwent conization for known lesions ranging from atypical squamous cells of undetermined significance (ASC-US) with high-risk HPV (hr-HPV) to high-grade squamous intraepithelial lesion (H-SIL/CIN2+) or adenocarcinoma. Two negative controls were included: one patient with post-menopausal bleeding and another from whom an inflammatory cervical sample was taken without conization. Prior to conization, a gynaecologist collected two cervical samples, fixed in PS or STM, from each patient. All but four cases were tested for panHPV (GP5+/GP6+) and specific hr-HPV subtypes (HPV16, 18, 31,33) by PCR using both media and all were processed for HC2. This study demonstrates that both HPV detection techniques work with PS, showing a specificity of 78.3% for HC2 and 92.8% for PCR compared to 83.8% for HC2 and 92% for PCR using STM. The efficacy of detecting HPV in PS-preserved H-SIL/CIN2+ was very high (96% for PCR using PS and 86% for HC2 using PS), which was in the same range as for PCR using STM, and which was only slightly lower than for HC2 using STM (96% and 89%, respectively). The differences were not statistically significant. It is concluded that ThermoFisher's PS is a valid liquid-based cytology medium for cervical samples, convenient for HPV testing by PCR with GP5+/GP6+ primers and by the HC2 assay.

Keywords: Cervical sample, conization, HPV, hybrid capture II, papspin, PCR, thermofisher
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Introduction

Cervical cancer is the second most common cause of death from cancer in women worldwide [1]. Human papillomavirus (HPV), when integrated in human DNA, is responsible for most of the cervical neoplasms [2].

The keystone for preventing cervical cancer development and detecting preneoplastic lesions is the Pap smear taken on a regular basis [3]. It has been shown that liquid-based cytology (LBC) has a sensitivity and specificity for detecting high-grade cervical intraepithelial neoplasia similar to the conventional Pap smear. However, the quality of preparation, more rapid microscopic interpretation, and the possibility to add molecular testing explains the fact that LBC has received much attention [4]. In recent years, different techniques have emerged, allowing the detection of the presence of HPV in LBC. These techniques have been advocated, in the European and French guidelines [5,6], as adjuncts to cytological analysis for the triage of lesions associated with atypical squamous cells of undetermined significance (ASC-US), atypical squamous cells, without the possibility of excluding a high-grade intraepithelial lesion (ASC-H) and atypical glandular cells (AGC).

The American Society for Colposcopy and Cervical Pathology has also updated their guidelines for HPV testing the follow-up of high-grade intraepithelial lesions (H-SIL) after treatment [7,8]. These recommendations are underlined in two meta-analyses by Arbyn *et al.* [9,10] who showed that

there is sufficient evidence in the literature to prefer HPV testing over repeat performance of Pap smears in the triage of women with atypical cytology and in the surveillance after treatment of cervical intraepithelial neoplasia (CIN) lesions [9,10].

The most reliable DNA-based tests now available are the consensus primer PCR assay and the Hybrid Capture II (HC2) microtitre assay, which is a hybridization technique using a single-stranded RNA probe and viral DNA without previous amplification [Hybrid Capture II; Digene Diagnostics, Gaithersburg, MD, USA] [11–13]. These techniques have been validated using the Surepath (Tripath Care Technologies, Burlington, NC, USA) [14,15] and Thinprep (Cytyc, Boxborough, MA, USA) [12] systems, but only the latter has been Food and Drug Administration approved for HC2 testing since 2002.

The present study aimed to validate ThermoFisher's Papspin (PS) for HPV testing by in-house PCR using GP5+/GP6+ primers and HC2 to compare the results with those obtained using Specimen Transport Medium (STM) (Digene Diagnostics, Gaithersburg, MD, USA).

Materials and Methods

Study group

The study involved 47 women (mean age, 40 years; range, 19–66 years). Forty-six of them underwent conization. One woman had only a cervical sample taken. In 45 cases, conization was planned for cytological and/or histological diagnoses, ranging from ASC-US with PCR-positive high-risk HPV (hr-HPV) to high-grade squamous intraepithelial lesion (H-SIL/CIN2+), AGC or adenocarcinoma.

The study included two negative controls, one of whom underwent conization for postmenopausal bleeding after a laparoscopically assisted supracervical procedure; an inflammatory cervical sample was taken from the other without conization.

Just before the conization procedure, all women had two Pap smears taken, one using a Cervex brush (Rovers Medical Devices, Oss, the Netherlands) that was immediately immersed in ThermoFisher's PS, and the other taken at random before or after the PS sample, using the sampling kit for the HC2 assay (STM).

Both specimens from each patient were split in our laboratory (Cliniques universitaires St Luc, UCL, Brussels) in order to obtain material for in-house PCR and HC2 analysis in both media. HC2 analysis was carried out in another university hospital [Université de Liége (ULg)] for technical reasons. The material was kept no longer than 2 weeks at room temperature before analysis, as specified by the manufacturers.

For each patient, a monolayer Pap smear was obtained by cytocentrifuging up to 4 mL of PS for cytological analysis after Papanicolaou staining. All patients had a classical work-up of their conization specimens after formalin fixation for 24 h. All four quadrants were included separately in paraffin-blocks, cut into 5-μm thick slices and stained with haematoxylin and eosin. Conization specimens were analysed independently of the cervical samples and the results of cytology and histology were pooled for final analysis.

All but four cases were tested for Pan HPV (GP5+/GP6+) and for specific hr-HPV genotypes (HPV 16, 18, 31, 33) by PCR in both media and all were processed for HC2. PCR and HC2 results were recorded independently.

PCR technique

DNA from cell suspensions was extracted (QIAMP DNA mini Kit; Qiagen, Valencia, CA, USA) according to the manufacturer's instructions using 5 mL of PS and 1 mL of STM, respectively. PCRs were performed with a 9700 thermocycler (PE Applied Biosystems, Norwalk, CT, USA) in a 25-μL reaction volume containing 1× PCR buffer, 3.5 mM $MgCl_2$, 1 U enzyme (Taq Polymerase; Applied Biosystems, Branchburg, NJ, USA), 0.25 mM dNTP (Roche Diagnostics, Mannheim, Germany), sense primers labelled with a fluorochrome (FAM or HEX) and antisense primers [11], both at 10 μM, and finally 5 μL of sample DNA. Table 1 gives the details of the technique for GP5+/GP6+ HPV testing and HPV 16, 18, 31, 33 genotyping.

PCR amplification was based on a 40-cycle thermal profile using denaturation and extension steps as described in

TABLE 1. Sequences of oligonucleotide primers, reaction conditions for PCR and PCR product characteristics

HPV subtype	Gene (DNA) region	Sense primer 5′- to 3′	Antisense primer 5′- to 3′	Annealing °C (s)	Extension °C (s)	Dye	Size (bp)
GP5+/GP6+	L1	gAAAAATAAACTgTAAATCATATTC	TTTgTTACTgTggTAgATACTAC	40 (120)	72 (90)	FAM	140
HPV-16	L1	CAAAATTCCAgTCCTCCA A	gCACAgggCCACAATAATgg	55 (45)	72 (60)	FAM	270
HPV-18	E7	AAgAAAAcgATgAAATAgATggA	ggCTTCACACTTACAACACA	55 (45)	72 (60)	HEX	102
HPV-31	E7	TTACCCgACAgCTCAgATgA	CACACgATTCCAAATgAgCC	52 (45)	72 (60)	FAM	190
HPV-33	E7	ATgAgAggACACAagCCAACg T	TgTgCCCATAAgTagTTgCT	52 (45)	72 (60)	HEX	260

HPV, human papillomavirus.