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Comparison of DR. HPV™ Chip Kit with hybrid capture II assay for the detection of human papillomavirus in clinical samples: a preliminary study

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Abstract. Human papillomavirus (HPV) is well known as an etiological factor for the development of anogenital carcinomas. The aim of our study was to compare the performance of USFDA approved Hybrid II (HCII) Assay and recently introduced DR. HPV™ Chip Kit for the detection of HPV DNA in clinical cervical scrapings from 40 patients. HPV DNA testing was performed using the automated HCII Assay system and DR. HPV™ Chip Kit. Taking cytological results as gold standard, it was found that HCII was more sensitive (36.4%) than DR. HPV™ Chip Kit (18.2%) although specificity was 100% with the latter method. In addition, both these molecular methods had comparable negative and positive predictive values. It was concluded that both HCII and DR. HPV™ Chip Kit have comparable specificity. However, sensitivity for detection of HPV in clinical samples with HCII is almost double as compared to DR. HPV™ Chip Kit.

INTRODUCTION

It is estimated that approximately 440,000 new cases of cervical cancers occur worldwide annually and about 80% of these cases occur in developing and under-developed countries (Masood, 1999). The role of human papillomavirus (HPV) as an etiological factor of these cancers has been established beyond any doubt. HPV is detected in >90% of reported cervical cancer cases (Walboomers *et al.*, 1999). HPV DNA sequences found in cervical carcinoma cell lines were the first clue to the role that high-risk types 16 and 18 play in altered cell growth (Schwarz *et al.*, 1985; Schneider-Gadicke & Schwarz, 1986). Further studies in cervical cancer cell lines have demonstrated many of the harmful effects of HPV in terms of cellular mutations (Havre *et al.*, 1995; Liu *et al.*, 1997) and genomic integrity (Hashida & Yasumoto, 1991; White *et al.*, 1994).

The essential component of any diagnostic test includes good sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). Screening for cervical cancer for many years has been based on Pap smear cytology, which suffers from subjectivity and depends on the skills of the observer. The Pap test usually shows variable sensitivities (30 to 87%) (Fahey *et al.*, 1995). Genotyping of HPV has undergone

tremendous developments over the years, allowing the detection of a wide range of HPVs. HPV type identification has become clinically useful for prognosis and risk type based-therapy (Cho *et al.*, 2003). Hybrid Capture II (HCII) assay by Digene, is the only United States Food and Drug Administration (USFDA) approved commercially available kit. HCII assay is a nonradioactive, immuno-chemiluminescence method that is based on the hybridization of genotype specific-RNA probes to the HPVs genomic sequence. Another newly available HPV DNA detection tool in the market is the DR. HPV™ Chip Kit, by DR. Chip Biotechnology. It is based on PCR-DNA microarray system, where the PCR products are hybridized to HPV type specific probes and results are based on the formation of specific spots for each type of HPV.

HCII Assay does not specifically identify each HPV genotypes, while DR. HPV™ Chip Kit clearly distinguishes genotypes of HPV. Moreover DR. HPV™ Chip Kit also allows multiple HPV infection detection. This preliminary study was aimed to assess the performance of DR. HPV™ Chip Kit as compared with that of FDA approved-Hybrid Capture II (HCII) Assay to detect HPV DNA in Malaysian cervical samples.

MATERIAL AND METHODS

Cervical scrapings from clinically suspected patients were collected from Obstetrics and Gynecology clinics of Hospital Universiti Sains Malaysia, Hospital Kota Bahru and Hospital Kuala Terengganu (n = 40). Clinically suspected patients were defined as females in the age group of 25 to 65, who were at higher risk of developing high-grade Cervical Intraepithelial Neoplasias (CIN 2 or 3) i.e. CIN 1 follow ups, smokers, women on contraceptive pills or with STDs. The specimens were collected using broom type collection device and are placed in Thin Prep Solution®. Liquid-based cytology does not require a smear of tissue transferred from sampler to slide. A cellular monolayer is produced that is free from the clumping and debris often seen in conventional smears. This was used for cytological examination while the remaining solution was used for HCII assay and DNA extraction for DR. HPV™ Chip Kit.

Pap smear preparations were performed and evaluation was performed by pathologists based on Bethesda reporting system 2001 (TBS 2001). These results were taken as the gold standard in comparing the two molecular techniques.

Hybrid Capture II

Residual swabs were kept at 4°C in ThinPrep® Test bottles. HPV DNA testing by the HCII assay method was performed with the automated HCII Assay system located in the Department of Pathology, Universiti Sains Malaysia, according to the protocol of the manufacturer. The samples were analyzed for the presence of High risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. HPV type 16 DNA (1 pg/ml) was used as a positive control. The HCII high risk HPV DNA cutoff of 1 pg/ml is equivalent to 100,000 HPV copies/ml or 5000 HPV copies per assay. Samples were classified as High risk HPV DNA positive if the relative light unit (RLU) reading obtained from the luminometer was equal to or greater than the mean value for the positive control as provided by the manufacturer. Single replicates were performed.

DR. HPV™ Chip Kit

For DR. HPV™ Chip Kit, which is based on polymerase chain reaction (PCR), DNA extraction was performed by conventional phenol/chloroform method. The integrity of the extracted DNA was checked by PCR with beta globin primers that were as follows:

B- GloRV 5' GAA GAG CCA AGG ACA GGT AC' 3
B- GloFW 5' CAA CTT CAT CCA CGT TCA CC'3

According to the manufacturer's protocol, a specific segment of gene from

HPV was amplified with primers provided in the kit.

PCR conditions

The Dr HPV Chip Kit-PCR was performed in 50 µl total reaction volume with 45 µl Dr HPV Chip Kit Master Mix and 5 µl of DNA template from clinical samples.

The amplification was performed in Eppendoff thermocycler with first denaturation step at 95°C for 5 min. Annealing temperature of 50 %C was used for 30 seconds and the primer extension at 72°C for 30 s. The final extension step was performed at 72°C for 7 min. These steps were repeated for an additional 34 cycles.

Hybridization with target probes

The products were hybridized with target probes pre-spotted on the wells in the chip. After the hybridization reaction, the hybridized target DNA was detected by enzymatic colorimetric development and the signal was captured and analyzed by DR. AiM™ Reader. High-risk types 16, 18, 31, 33, 45, 58 and low-risk types 6, 11, 34 and 70 were identified with this type. The Statistical Analysis was performed by SPSS version 11 to compare the sensitivity and specificity of the two methods.

RESULTS

The concordance of HPV detection results for the 40 study subjects, using cytology, DR. HPV™ Chip Kit and HCII Assay is depicted in Table I, Table II and Table III.

Table I shows that only two samples (5%) positive DR. HPV™ Chip Kit were also suspected positive for cytology. In addition, nine samples negative with DR. HPV™ Chip Kit were seen to have cytopathological changes suggestive of viral infection. So in total 11 samples (27.5%) were positive with cytology but only 2 (5%) were positive with DR. HPV™ Chip Kit.

Figure 1 shows the result of Dr HPV Chip Kit PCR of Sample DNA. The amplicon size were compared with a 100 bp DNA ladder. An amplicon size of 450bp indicates a positive amplification as seen in lane 2, 3 and 6.

Five samples (12.5%) were positive with HCII Assay while 11 samples (27.5%) were reported to have cytopathological features of viral infection as shown in Table II. However, seven samples negative by HCII Assay were suspected positive with cytology. In total, 29 (72.5%) samples were found negative for any viral changes by cytopathological examination while 35 (87.5%) were tested negative by HCII Assay.

The results of DR. HPV™ Chip Kit with HCII Assay are compared in Table III. Only two samples were positive with DR. HPV™ Chip Kit (5%) while five samples (12.5%) were positive with HCII Assay. From the two HPV-positive cases by DR. HPV™ Chip Kit, one was genotyped to be HPV-16 while the other was HPV-58. In total, 35 samples (87.5%) were negative by HCII Assay while 38 (95%) were negative by DR. HPV™ Chip Kit.

The sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) for DR. HPV™ Chip

Table 1. HPV detection results of DR. HPV™ Chip Kit and cytology

DR. HPV™ Chip Kit		Cytology		
		POS	NEG	TOTAL
	POS	2	0	2
	NEG	9	29	38
	TOTAL	11	29	40

Table 2. HPV detection results of HCII assay and cytology

HCII Assay		Cytology		
		POS	NEG	TOTAL
	POS	4	1	5
	NEG	7	28	35
	TOTAL	11	29	40

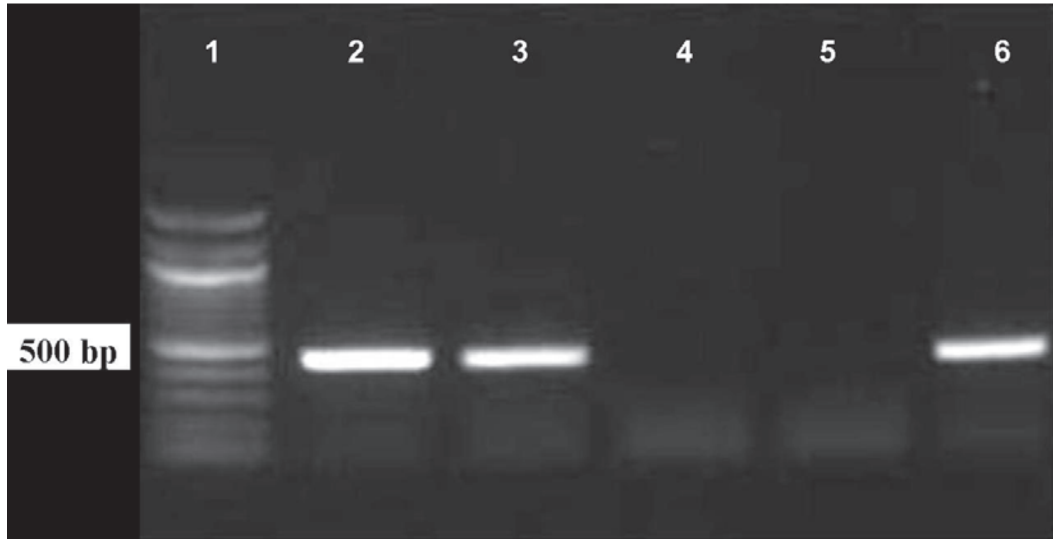

Figure 1. Result of Dr HPV Chip Kit PCR of Sample DNA. Lane 1: 100 bp DNA ladder. Lane 2: Positive control. Lane 3 & 6: Positive amplification of size 450bp.

Table 3. HPV detection results of DR. HPV™ Chip Kit and HCII assay

DR. HPV™ Chip Kit		HCII Assay		
		POS	NEG	TOTAL
	POS	2	0	2
	NEG	3	35	38
	TOTAL	5	35	40

Table 4. Comparison of Sensitivity, Specificity, NPV and PPV

	HCII Assay	DR. HPV™ Chip Kit
Sensitivity	36.4%	18.2%
Specificity	96.6%	100.0%
NPV	80.0%	76.3%
PPV	80.0%	100.0%

Kit and HCII assay in comparison to cytology as the gold standard is displayed in Table IV.

DISCUSSION

The World Health Organization has indicated that cervical cancer is the second cause of malignant neoplasia and death in women worldwide (Munoz, 2000). Similar trend is also seen in Malaysia where the incidence of this cancer is 12.9 per 100,000 (Registry, 2003). The reliability of screening for cervical neoplasia using a Papanicolaou smear alone is questionable and thus newer molecular methods are now being used to improve the accuracy of routine diagnosis of cervical lesions. The hybrid capture assay has proven a reliable, accurate and reproducible HPV test method for routine clinical practice (Bozzetti *et al.*, 2000).

Overall, sensitivity of HCII Assay in detecting High-risk HPV positive cases was found to be higher (36.4%) than the DR. HPV™ Chip Kit (18.2%). This was expected as the number of high-risk HPV types included in the DR. HPV™ Chip Kit is only 8 compared to the 13 types detectable by the HCII assay. These two HPV DNA detection tests gave an overall kappa value of 0.538 (95% confidence intervals, CI) and the results were statistically significant ($p< 0.000$)

Cases that were suggestive of viral infection by cytology but eventually turned out to be negative by either DR. HPV™ Chip Kit or HCII assay could be because cytology is an observer-biased method and the results are based entirely on subjective interpretation. Moreover, as cytology cannot identify HPV in particular, the features suggestive of viral infection could also have been due to other viruses e.g. HSV, which were not detected by either HCII or DR. HPV™ Chip Kit.

Though HCII was found to be more sensitive than DR. HPV™ Chip Kit, its specificity was found to be 100%. This result was contrary to that found by Hantz *et al*. (2005) who compared Hybrid Capture II with HPV Consensus kit (Argene) and found the kit more sensitive than HCII assay (Hantz *et al.*, 2005). Other studies, which were done to compare HCII with PCR based methods, have found these methods almost similar in sensitivity (Bozzetti *et al.*, 2000; Chatterjee *et al.*, 2003; Nonogaki *et al.*, 2004). In our study, NPV of DR. HPV™ Chip Kit was found to be slightly lower than HCII. However, its PPV was found to be 100%. We found that HPV detection by DR. HPV™ Chip Kit was more convenient since it does not require any visual-aiding devices as compared to HCII assay where a luminometer is used in interpreting the result. In addition, HCII lacks the ability to discriminate specifically each genotype of high-risk HPVs.

In conclusion, HPV detection by DR. HPV™ Chip Kit and HCII Assay showed good agreement. Both were low in sensitivity as compared to cytology but this was compensated by high level of specificity. In addition, these molecular methods had comparable negative and positive predictive values. The limitation of this study was a relatively small number of samples used, thus a larger study would enable further evaluation of DR. HPV™ Chip Kit.

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