CHAPTER I
INTRODUCTION

Human papillomavirus (HPV) is common sexually transmitted pathogen that cause genital wart and anogenital cancer in man and woman. Infection of the cervix with high-risk HPV is accepted to be causally associated with the development of cervical cancer, the second most common cancers in woman worldwide, with an annual incidence of almost half a million and mortality rate of approximately 50%. The development of cervical cancer is considered to be a multistep process, where HPV is necessary but in itself an insufficient cause. Cervical cancer is a rare complication of infection with high-risk HPV, but every abnormal or dysplastic lesion of the cervix is potentially malignant and may develop into cervical cancer at any time. Disease can only develop when there is persistent HPV infection of the cervical epithelium but can be a largely preventable disease through the detection, treatment and follow-up of its precursors. Traditionally, this has been accomplished through screening woman with cervical cytology, and referring woman with abnormal cytology for colposcopy, histological sampling and treatment (1).

Due largely to the effective cervical cancer screening by the conventional "Papanicolaou" (Pap) smear technique, the incidence and death rate of invasive cervical cancer are markedly decreasing in developed countries. However, the false-negative results due to error in sampling, interpretation or follow-up are limitations of this method and some women with negative tests continue to develop cervical cancer. Liquid-based cytology (LBC) has emerged to improve these problems although it is relatively expensive for most developing coutries. Besides both Pap smear and LBC, cytology-based screening method, require well-trained cytotechnologists and cytopathologists that are lacking in these coutries (2). And because of the difficulties of propagating HPV virus in tissue culture and the unreliability of serological tests to diagnose HPV infection, the detection of viral deoxyribonucleic acid (DNA) of HPV infection relies on DNA testing as either an alternative or adjunctive test to cytology for the detection of cervical cancer and its precursors. Since all HPV types are closely related, assays can be designed to target conserved regions of the genome (3).

In large trials and epidemiological studies have been validated by two methods. One of the methods is the Hybrid Capture II® (HC2®, Digene Corporation, Gaithersburg, USA) that is commercially based on the hybridization of the target HPV DNA to labeled RNA probes in solution. The method is able to be detected 13 high-risk of HPV DNA (HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and 5 low-risk types (HPV6, 11, 42, 43, and 45). This assay has become the standard in laboratories for many countries, is widely used in clinical studies, and has FDA approval. Although HC2® has some limitations include unidentifying of specific HPV genotypes, less sensitivity than polymerase chain reaction (PCR) method and cross-reactivity of probe cocktails (4-6). And the other is PCR-based assays that are widely developed, have the highest sensitivity, require very small amounts of specimen and can much more identify genotypes of HPV. The sensitivity and specificity of these methods vary, depending on the primer sets, the size of the PCR product, reaction conditions, the spectrum of HPV DNA amplified, and the ability to detect multiple types (3). Especially, consensus or general primers are widely used to amplify a broad-spectrum of HPV genotypes in the late (L) 1 region, most conserved part of the genome, such as PGMY09/11, GP5+/6+, and SPF_{10} that can be generated 450, 150, and 65 base pairs (bps) in length of PCR products, respectively. After amplification, the amplified products may be performed by gel electrophoresis, restriction enzyme analysis, dot blot or line strip hybridization, or sequencing (1, 3).

Several commercial PCR-based assays are available in the present. Such as AMPLICOR® HPV Test (Roche Diagnostics, Meylan, France) can detect the same high-risk HPV types by a broad-spectrum PCR but more sensitive and high throughput of microtiter format than HC2® although identifying of HPV genotypes that is unavailable (1, 7). For INNO-LiPA HPV Genotyping v2 test (Innogenetics, Ghent, Belgium) and Linear Array HPV Genotyping test (LA-HPV; Roche Molecular Systems Inc, Pleasanton, Calif, USA) were independent reverse hybridization assays for the identification of HPV genotypes by using type-specific probe, after positive samples were amplified by using biotinylated SPF_{10} and PGMY09/11 primers, respectively. Both reverse hybridization assays permit the identification of 25 and 37 different HPV genotypes, respectively (1, 8-13).

Although the PCR-based methods have several advantages in diagnostic purpose especially the highly sensitivity and specificity as well as its rapidity and high throughput but almost all of them are available commercially with an expensive cost especially the method can identify the genotypes of HPV. This is a major limitation of the assays in diagnostic application. Standard laboratory diagnosis for screening of cervical cancer is based mainly on Pap smear although it is relatively inexpensive and good specificity but several limited in sample processing, sensitivity and human resources. In order to get an accurate diagnosis of HPV infection, we developed the rapid, easily performed, and inexpensive TaqMan-based real-time PCR and REA for detection and typing of HPV DNA in cervical specimen.

Aims of the study

1. To develop TaqMan-based real-time PCR technique for detection of HPV DNA.
2. To develop restriction enzyme analysis technique for HPV genotyping.
3. To compare the HPV DNA detection rate in cervical samples with VIA positive and negative by using real-time PCR optimized in this study.