



UNIVERSITI PUTRA MALAYSIA

***PREVALENCE OF HUMAN PAPILLOMA VIRUS INFECTION AND ITS
ASSOCIATED RISK FACTORS AMONG NON-CERVICAL CANCER
WOMEN IN SELANGOR, MALAYSIA***

NURUL ASYIKIN BINTI ABDUL RAHMAN

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ASSOCIATED RISK FACTORS AMONG NON-CERVICAL CANCER WOMEN
IN SELANGOR, MALAYSIA**

By

NURUL ASYIKIN ABDUL RAHMAN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra
Malaysia, in Fulfilment of the Requirements for the Degree of Master of
Science
October, 2009**



DEDICATION

*This thesis is dedicated to all of the following people who have
inspired me in a special way of my life:*

My beloved parents- for your support

My husband- for the courage and eternal love

My childrens- for reminding me not to give up

My friends- for being there

My supervisor and co supervisor – for trusting me

*“Desire is the key to motivation, but it’s the determination and
commitment to an unrelenting pursuit of your goal- a commitment to
excellence – that will enable you to attain the success you seek”*

Mario Andretti

Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirements for the degree of Master of Science

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NURUL ASYIKIN BINTI ABDUL RAHMAN

October, 2009

Chair: Dr Chong Pei Pei, PhD

Faculty: Faculty of Medicine and Health Sciences, UPM.

Persistent high-risk human papillomavirus (HPV) infection is known to play an important role in the carcinogenesis of cervical cancer. Since new intervention strategies namely improved HPV testing as a screening programme and HPV vaccination have been aggressively promoted in the past few years, it is crucial to discover the HPV distribution in Malaysia in order to maximize the cost-effectiveness of the intervention strategy. Hence, this study was conducted to assess the pattern of HPV type distribution in Southern Selangor. This study was also conducted to determine the behavioural and sexual lifestyle as well as the socio-demographic factors that contribute to HPV infections among non-cervical

cancer women in South Selangor. In this study, the efficiency of Seeplex HPV kit was also compared with nested PCR by measuring the sensitivity and also the specificity. A total of 200 cervical swab samples were collected from women attending Obstetrics and Gynecology Clinics in several hospitals in Selangor. Informed consent was obtained from each participant, who was required to answer a self-administered questionnaire regarding her socio-demographic details as well as sexual history and lifestyle. The HPV DNA was detected via nested PCR using the MY09/MY11 primers as outer primer pair and GP5+/GP6+ as inner primers that target the L1 gene of the viral genome. The PCR products were subjected to automated DNA sequencing to determine the HPV genotype. Forty samples were amplified using Seeplex HPV kit by amplification of target DNA based on DPO™ (Dual Priming Oligonucleotide) technology. Out of the 200 samples collected from March 2007 until August 2007, 84(46.7%) were detected through nested PCR as positive for HPV DNA. The most common HPV type found was type 16 (85.7%), followed by HPV type 18 (7.1%), HPV 33 (3.6%), HPV 31 (1.2%), HPV 58 (1.2%) and HPV 87 (1.2%). HPV types 16, 18, 31, 33 and 58 are high-risk types associated with development of cervical cancer, whereas HPV 87 is thought to have low to intermediate risk. The prevalence of HPV infection was found to be the highest in the 30-45 age group (58.3%), followed by <30 years old (21.4%) and >46 years old (20.2%). Patients who have spouses or partners with secondary educational level were found to have higher risk of getting HPV infections with an odds ratio of 1.5 (95% CI 0.84-2.72) compared to those with primary and tertiary educational level. Malay women

were found to be more likely to get HPV infection with OR = 1.5 (95% CI 0.82-2.75) compared to Chinese and Indian. Women who were suffering from gynecological problems were also found to have 2.5 times (95% CI 1.0-6.6) higher chances of getting HPV infection as compared with women who were not suffering from any gynecological problems. This study had shown that nested PCR is highly sensitive in detecting HPV DNA even in low risk patients as compared to Seeplex HPV kit. The sensitivity and specificity of nested PCR were 100% and 83.3% respectively, whereas the sensitivity and specificity of Seeplex HPV kit was 40% and 100%.

Keywords: Human papillomavirus (HPV), cervical cancer, Malaysian women.

Abstrak thesis yang dikemukakan kepada senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Sarjana Sains

**KELAZIMAN JANGKITAN OLEH KUMAN HUMAN PAPILLOMAVIRUS (HPV)
DAN FAKTOR-FAKTOR RISIKO YANG MEMPENGARUHI JANGKITAN
DIKALANGAN WANITA YANG TIDAK MENGIDAP KANSER SERVIK DI
SELANGOR, MALAYSIA.**

Oleh

NURUL ASYIKIN BINTI ABDUL RAHMAN

OKTOBER, 2009

Pengerusi: Dr. Chong Pei Pei, PhD

Fakulti: Perubatan dan Sains Kesihatan

Jangkitan HPV berisiko tinggi secara berterusan diketahui memainkan peranan yang penting dalam karsinogenesis kanser servik. Sejak satu kaedah baru yang telah diperbaharui telah diperkenalkan sebagai program “screening” dan vaksinasi HPV dipromosikan secara besar-besaran, adalah penting untuk mengetahui dengan tepat tentang taburan HPV di Malaysia untuk mengoptimumkan keberkesanan langkah-langkah pencegahan dan rawatan. Oleh itu, kajian ini telah dijalankan untuk mengesan jenis-jenis HPV dalam taburan penduduk di Selatan Selangor. Kajian ini juga telah dijalankan untuk mengenalpasti gaya hidup dan faktor-faktor sosio-demografik yang menyumbang kepada jangkitan HPV di kalangan wanita-wanita yang tidak menghidap kanser servik di Selatan Selangor. Di dalam kajian ini juga keefisien Seeplex HPV kit telah diuji dan dibezakan dengan kaedah “nested PCR” yang

telah digunakan dengan menguji sensitiviti dan spesifisiti. Dua ratus sampel dari pengelap kapas telah di ambil daripada wanita-wanita yang mendapatkan rawatan di Klinik Obstetrik dan Ginekologi di beberapa hospital di Selatan Selangor. Setelah mendapatkan kebenaran daripada wanita-wanita yang berminat untuk menjadi responden, mereka telah diminta untuk menjawab soalan soal-selidik yang telah diberikan berkaitan dengan sosio-demografik dan sejarah seksual dan juga gaya hidup mereka. DNA HPV telah dikesan dengan menggunakan kaedah “Nested PCR” yang menggunakan primer luaran MY09/MY11 dan GP5+/GP6+ sebagai primer dalaman yang mensasarkan L1 gen daripada genom virus HPV. Sampel yang positif di hantar untuk menjalani kaedah “Sequencing” secara automatik bagi menentukan genotip HPV yang terlibat. Empat puluh sampel telah dipilih untuk melalui kaedah Seeplex HPV Kit yang mensasarkan DNA HPV melalui kaedah PCR juga berdasarkan teknologi DPO™ (Dual Priming Oligonucleotide). Lapan puluh empat orang daripada dua ratus orang (46.7%) responden telah didapati positif dan mengandungi DNA virus HPV. Jenis HPV yang paling banyak dikenalpasti di kalangan responden yang positif di dalam kajian ini adalah HPV jenis 16 (85.7%) diikuti oleh jenis HPV jenis 18 (7.1%), HPV jenis 33 (3.6%), HPV jenis 31 (1.2%), HPV jenis 58(1.2%) dan HPV 87 (1.2%). Telah diketahui bahawa HPV jenis 16,18, 31,33 dan 58 adalah merupakan HPV jenis berisiko tinggi yang boleh yang menyumbangkan kepada terjadinya kanser servik manakala HPV jenis 87 diklassifikasikan sebagai jenis yang berisiko rendah kepada sederhana. Kelaziman jangkitan HPV telah ditemui paling banyak pada golongan responden yang berumur 30-45 tahun (58.3%) diikuti oleh mereka yang berumur kurang

dari 30 tahun (21.4%) dan lebih daripada 46 tahun (20.2%). Responden yang mempunyai suami atau pasangan yang mempunyai tahap pendidikan kelas menengah didapati odds rationya 1.5 (95% CI 0.84-2.72) kali lebih tinggi berisiko untuk mendapat jangkitan HPV berbanding mereka yang mempunyai suami atau pasangan yang mendapat pendidikan rendah dan tinggi. Wanita berketurunan Melayu di dalam kajian ini dikenalpasti berisiko 1.5 kali untuk mendapat jangkitan HPV dengan bacaan Odd Rationya $OR = 1.5$ (95% CI 0.82-2.75) berbanding responden yang berketurunan Cina dan India. Wanita yang mengalami masalah ginekologi adalah didapati mempunyai 2.5 kali (95% CI 1.0-6.6) lebih berisiko untuk mendapat jangkitan HPV berbanding mereka yang tidak mempunyai masalah ginekologi. Kajian ini telah mendapati teknik nested PCR adalah terlalu sensitif di dalam mengesan DNA virus HPV walaupun pada responden yang mempunyai risiko yang rendah kepada jangkitan berbanding menggunakan teknik Seeplex HPV kit. Sensitiviti dan spesifisiti nested PCR telah didapati 100% dan 83.3% manakal sensitiviti dan spesifisiti Seeplex HPV kit adalah 40% dan 100%.

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This thesis was submitted to the senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Chong Pei Pei, PhD

Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Rusinahayati Mokhtarudin, MBBS, MOG

Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

Rozita Rosli, PhD

Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

Maha Abdullah, PhD

Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

HASANAH MOHD GHAZALI, PhD

Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 11 February 2010

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution.

NURUL ASYIKIN ABDUL RAHMAN

Date:

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LIST OF ABBREVIATIONS

ASCUS	Atypical Squamous Cells of Undetermined Significance
bp	Base-pair
kb	Kilobase-pair
β	Beta
®	Registered
°C	Degree of Celcius
%	Percentage
μ l	Microliter
ml	Mililiter
mg	Miligram
mM	Milimolar
pmol	Picomolar
CC	Cervical Cancer
CI	Confidence Interval
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleic triphosphate
DPO	Dual Priming Oligonucleotide
<i>et al.</i>	Et alii
FDA	Food and Drug Administration
HIV	Human Immunodeficiency Virus
HPV	Human Papillomavirus



HM	HPV Marker
HR-HPV	High Risk Human Papillomavirus
HSIL	High Grade Squamous Intraepithelial Lesions
IARC	International Agency for Research on Cancer
Ig	Immunoglobulin
L	Ladder
LR-HPV	Low Risk Human Papillomavirus
LSIL	Low Grade Squamous Intraepithelial Lesions
MAKNA	National Cancer Council
MgCl ₂	Magnesium Chloride
Min	Minute
NK	Natural Killer
NPV	Negative Predictive Value
OC	Oral Contraceptive
OR	Odd Ratio
p53	Tumour protein 53
PBS	Phosphate Buffer Saline
PCR	Polymerase Chain Reaction
PPV	Positive Predictive Value
rpm	Revolutions per minute
SD	Standard Deviation
Sec	Second
SPSS	Statistical Package for the Social Sciences



STD	Sexually Transmitted Disease
STI	Sexually Transmitted Infection
TAE	Tris acetate EDTA buffer
T _m	Melting Temperature
™	Trade Mark
w/v	Weight over volume
WHO	World Health Organisation



CHAPTER 1

INTRODUCTION

The human papillomaviruses (HPVs) are DNA double stranded and small- size (approximately 8000 base pairs) viruses that have cohabited with the human species over dozens of millennia, suffering relatively few changes in their genetic composition. Human papillomaviruses have adjusted to changing environments through point mutations rather than recombination with other papillomaviruses (Chan *et al.*, 1995). HPVs encode two proteins, E6 and E7 that are important for cell immortalization (Vousden, 1994). E6 and E7 interact with the tumour suppressor proteins p53 and pRb, respectively. The actual mechanism on how the Rb and the p53 genes degraded is complex. The E6 proteins inactivates p53 gene and E7 proteins bind with the Rb gene products causing inactivation of these tumour suppressor genes. When these genes are inactivated, cell with damaged DNA content are allowed to enter the cell cycle thus generating new clones of cell, which are abnormal (dysplastics).

One of the most important discoveries in the etiologic investigation of cancer over the last 25 years had been the demonstration that cervical cancer was caused by the persistent infection by certain genotypes of Human Papillomavirus (HPV). HPV 16 is the most prevalent type found in women with cervical cancer worldwide, and together with HPV 18, 31, and 45 accounts for about 80% of invasive cervical cancer. There is now very well established evidence for a



causal association between infection with certain types of HPV and the development of cervical cancer. Evidence from molecular studies have identified mechanism by which high risk types of HPV contribute to carcinogenesis, while epidemiological studies indicated that the HPV can be recovered from more than 95% of all cervical tumours (Cuzick *et al.*, 1998).

Infection by HPV is basically a sexually transmitted disease. As such, both men and women are involved in the epidemiological chain of infection and are able at the same time to be asymptomatic carriers, transmitter and also the victims of the infection by HPV. In this sense, the risk factors associated with the infection by HPV are clearly related to the individual's sexual behaviour. The most important are: early age at first sexual relationships, high number of sexual partners throughout life, sexual contacts with high risk individuals (in men, frequent contact with women that practise prostitution and in women, frequent contacts with men with multiple sexual partners). High prevalence groups can be identified socially in the population of women who practise prostitution and in persons infected by the Human Immunodeficiency Virus (HIV).

The prevalence of HPV infection is highest in the African continent at 23%, with 15.6% in the Americas, 8.3% in Asia, and 6.6% in Europe (Castellasague *et al.*, 2007). Current estimates show that approximately 20 million people are infected with HPV in the United States (Cates, 1999). In 2005, an estimated of 16,370 new infections and 3710 deaths occurred (American Cancer Society 2006). This



important to emphasize that at young ages and at the most sexually active ages in spite of very frequent infection by HPV, the great majority of the infected women (more than 90%) resolve the infection spontaneously and the infection persist in only small fraction of women (Elfgren *et al.*, 2000). It is a small group of women, chronic carriers of high-risk HPVs, who have a high risk of progression and development of neoplastic lesions of the anogenital tract (Schlecht *et al.*, 2001).

Cancer, in general, will remain the number one concern of the human population worldwide. Cervical cancer is the second most common malignancy among women worldwide, with close to 500,000 new cases each year and close to half the number (250,000) of deaths annually (Parkin *et al.*, 2005, Pisani *et al.*, 1999). Although cervical cancer occurs across the globe, its frequency is unevenly distributed (Yang *et al.*, 2004). Most cervical cancer, approximately 80% of all cases, occurs in developing countries. In these countries, cervical cancer is the most predominant cancer in women. Cervical cancer cases in developed countries are less frequent. The bulk of cervical cancer is in Asia emerges in underdeveloped or developing countries, with nearly 330,000 cases. In Malaysia, cancer of the cervix is the second most common cancer among females after breast cancer. The incidence of this cancer is 11.6 per 100,000 population, with the age standardized rate of 16.2 per 100, 000 (Ministry of Health, Malaysia, 1999). Malaysia has introduced Pap smear since late sixties and early seventies; however, there is no change in the pattern of the prevalence of this cancer



indicating that the Pap smear coverage in the country has not achieved the targeted population at risk.

Conventional Pap smear will remain the main screening method for this cancer as it has been proven to reduce the incidence by 43%. However, the sensitivity of Pap smear varies from 30-87%. It is time to move forward and embrace molecular Pap smear (Stoler *et al.*, 2001). Establishment of HPV infection status and identification of the HPV genotype in clinical samples are gaining ground as important prognostic indicators in the clinical screening of women and management of those found to be at risk for the development of cervical cancer. Testing for HPV DNA is now recommended for most women with equivocal findings on Pap smear analysis. Combining the existing Pap smear analysis protocol with HPV screening has been proposed as a more definitive method for assessing the risk of an individual to develop cervical cancer (Ledger *et al.*, 2000; Burd, 2003). The development of two prophylactic vaccines, Cervarix and Gardasil, are already in a very advanced phase of implementation at the world level, and both vaccines have demonstrated not only safety and immunogenicity but also efficacy for the prevention of cervical neoplastic lesions as well as vaginal, vulval and genital warts for Gardasil.



Since there is no data on the prevalence of specific HPV genotypes that have been published in Malaysia, this study was aimed to:

1. Determine the prevalence of HPV infection and genotype distribution among non-cervical cancer women in Selangor, Malaysia. It is important to have relevant baseline data to identify viral types that are spreading through the population to gauge the efficiency of the new vaccines.
2. Identify socio-demographic factors as well as behavioral and sexual lifestyle that associated with high-risk HPV infection in our population.
3. Evaluate a novel test (The Seeplex® HPV Genotyping kit) developed by Seegene USA, to improve the sensitivity of detection of high-risk HPV-DNA, and to allow high throughput testing. This was assessed by comparing the accuracy of Seeplex® HPV genotyping (Seegene, USA) PCR kit with conventional nested PCR and then measure sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of both methods.



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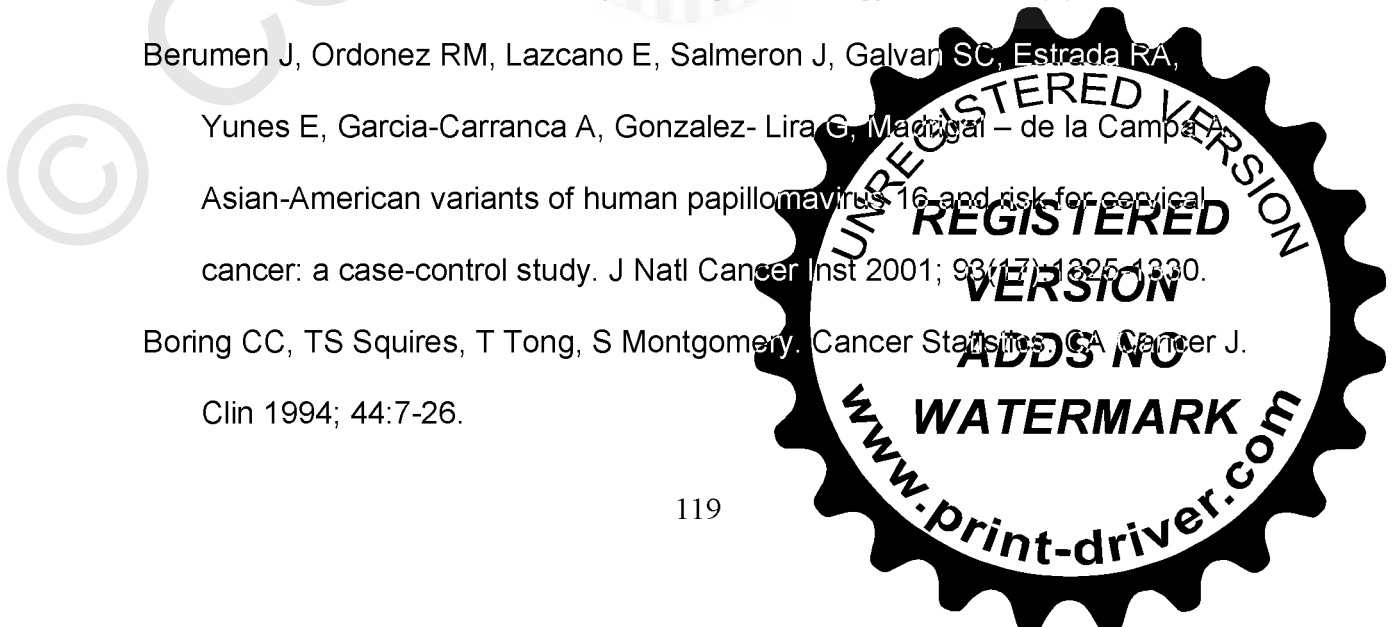
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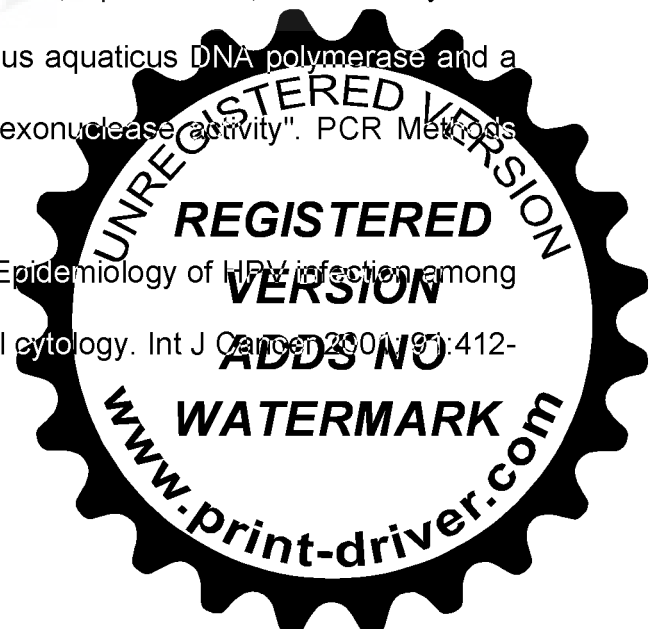
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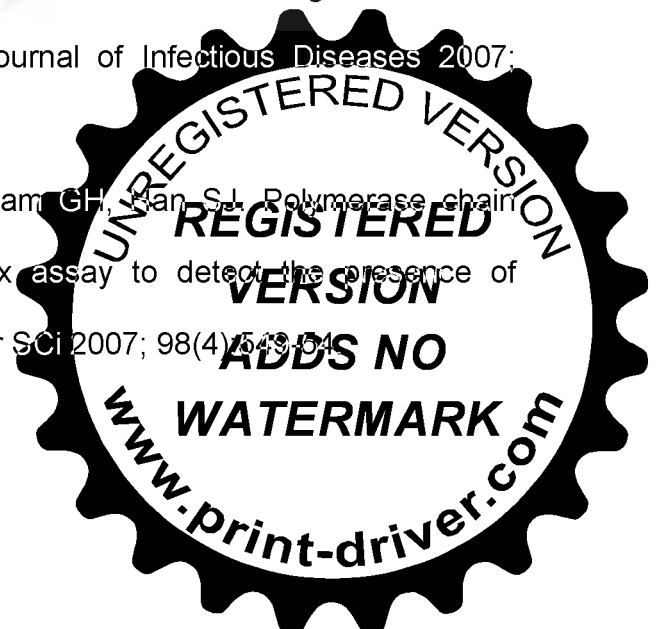
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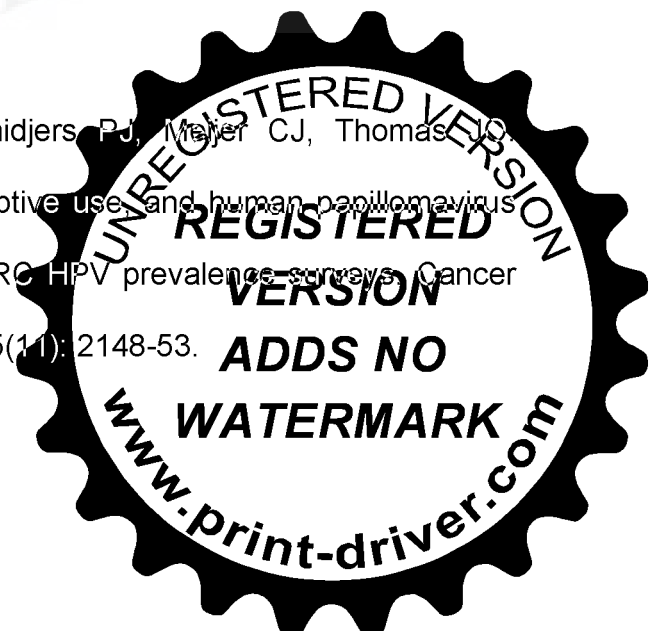
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