

การตรวจ Human papillomavirus type 16 โดยการรวมกลุ่มอนุภาคในทองคำ



นางสาวกมลวรรณ พลายงาม

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DETECTION OF HUMAN PAPILLOMAVIRUS TYPE 16 BY GOLDNANOPARTICLE
IMMUNOAGGLUTINATION

Miss Kamonwan Phlaingam

A Thesis Submitted in Partial Fulfillment of the Requirements
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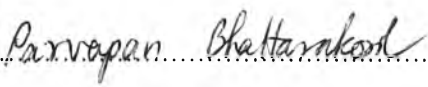
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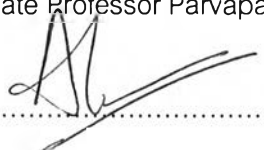
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กมลวรรณ พลายงาม : การตรวจ Human papillomavirus type 16 โดยการรวมกลุ่มอนุภาคนาโนทองคำ. (DETECTION OF HUMAN PAPILOMAVIRUS TYPE 16 BY GOLDNANOPARTICLE IMMUNOAGGLUTINATION) อ. ที่ปรึกษาวิทยานิพนธ์หลัก : รศ.ดร. ภาวพันธ์ ภัทรโกศล,อ. ที่ปรึกษาวิทยานิพนธ์ร่วม อ.นพ. ดร.อมรพันธ์ เสรีมาศพันธ์, 103 หน้า.

ในปัจจุบันพบว่ามะเร็งปากมดลูกเป็นมะเร็งอันดับสองที่พบได้ในผู้หญิงทั่วโลก ประมาณ 470,000 คนต่อปี สาเหตุของการเกิดและการพัฒนาเป็นมะเร็งปากมดลูกมีความเกี่ยวข้องกับ การคงอยู่ ของการติดเชื้อกลุ่มเสี่ยง HPV โดยเฉพาะชนิด 16 และ 18 ซึ่งปัจจุบันการตรวจคัดกรองหามะเร็งปากมดลูก แบ่งได้สองวิธีหลักคือ การตรวจความผิดปกติของเซลล์ปากมดลูกและการตรวจหา HPV DNA อย่างไรก็ตามได้มีความพยายามที่จะพัฒนาวิธีการตรวจแบบใหม่ขึ้น และในการศึกษานี้ได้พัฒนาการตรวจ HPV 16 แอนติเจน โดยอาศัยอนุภาคนาโนทองคำ โดยวิธีนี้จะเชื่อมต่อกับอนุภาคนาโนทองคำด้วยแอนติบอดีต่อ HPV16L1 หรือ HPV16E6 เพื่อตรวจหาแอนติเจน HPV16L1 หรือ HPV16E6 ซึ่งสามารถอ่านผลการทดสอบได้จากการตกตะกอนของปฏิกิริยาที่เกิดขึ้น

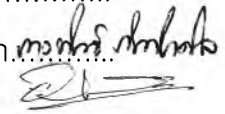
ในการทดสอบครั้งนี้ใช้ตัวอย่างทั้งหมด 40 ตัวอย่างจากผู้ป่วยที่ผลการตรวจทางพยาธิวิทยาปกติ, ผิดปกติในระดับ LSIL, HSIL และมะเร็ง ซึ่งตัวอย่างทั้งหมดจะทำการทดสอบหา HPV16 ด้วยวิธีที่พัฒนาขึ้นใหม่เปรียบเทียบกับวิธี type-specific PCR ผลการทดสอบพบว่า มี 25 ตัวอย่างที่ตรวจพบ HPV16 DNA ด้วยวิธี type-specific PCR เมื่อตรวจด้วยวิธี immunogoldagglutination ที่พัฒนาขึ้นพบแอนติเจน HPV16L1 ร้อยละ 60(15/25) และ พบแอนติเจน HPV16E6 ร้อยละ 36(9/25) ดังนั้นความไวของการทดสอบการตรวจแอนติเจน HPV16L1 ด้วยวิธีใหม่นี้คิดเป็นร้อยละ 60(15/25) และ แอนติเจน HPV16E6 ร้อยละ 36(9/25) ซึ่งต่ำกว่าวิธี type-specific PCR อย่างไรก็ตามพบว่าในตัวอย่างที่มีผลทางพยาธิผิดปกติในระดับสูง (HSIL) จนถึงเป็นมะเร็งพบว่าความไวของการทดสอบหา HPV16E6 เพิ่มขึ้นเป็นร้อยละ 69.23(9/13) แสดงว่าการตรวจแอนติเจน HPV16E6 ด้วยวิธี immunogoldagglutination assay อาจใช้เป็นวิธีการตรวจคัดกรอง ผู้ป่วยระยะก่อนเป็นมะเร็งและระยะเป็นมะเร็งได้

สาขาวิชา จุลชีววิทยาทางการแพทย์

ปีการศึกษา 2552

ลายมือชื่อนิสิต.....กมลวรรณ..... ภาวพันธ์.....

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....อมรพันธ์.....

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ADVISOR : ASSOC.PROF.PARVAPANBHATTARAKOSOL, Ph.D., THESIS CO-

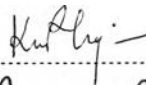
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Cervical cancer is the second leading cause of cancer deaths in women worldwide. More than 470,000 cases are diagnosed each year. Development of cervical cancer is associated with persistent of high-risk HPV infection especially type 16 and 18. Now, methods for screening cervical cancer can be divided into detection of cervical cell dysplasia and detection of HPV DNA. Several attempts in developing a new diagnostic method have been made. To accomplish this objective, the optical detection assay of HPV type 16 antigens using goldnanoparticle based on immunoagglutination was developed. The assay used AuNPs conjugated with either HPV-16L1 or HPV-16E6 polyclonal antibodies for detecting HPV-16 L1 or E6 proteins directly from clinical specimens and the result was visibly detected by an agglutinate of the reaction.

In this study, a total of 40 samples obtained from patients with normal-pathology, LSIL, HSIL and CaCx, were detected of HPV-16 by the newly assay and type-specific PCR. The result showed that 25 samples were detected with HPV-16 by type-specific PCR. All 25 HPV-16 samples were positive for HPV16L1 60% (15/25) and HPV16E6 36% (9/25). Therefore, the sensitivity of the assay was 60% (15/25) for HPV-16L1 detection and 36%(9/25) for HPV-16E6 detection which is sensitive less than detection by type specific PCR. However, in high grade CIN3 and CaCx samples sensitivity of the assay for detection HPV-16E6 was reached to 69.23% (9/13). These results indicated that the immunogoldagglutination assay for detect HPV16E6 might be an appropriate for screening cervical pre cancerous and cancerous patients.

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LIST OF ABBREVIATIONS

ADDL	=	Amyloid β -derived diffusible ligand
APS	=	Ammonium persulfate
ASC	=	Atypical squamous cell
ATCC	=	American Type Culture Collection
Au	=	Gold
AuNPs	=	Gold nanoparticles
CaCx	=	Cervical cancer
CIN	=	Cervical intraepithelial neoplasia
CSF	=	Cerebrospinal fluid
DB	=	Dot blot
DNA	=	Deoxyribonucleic acid
DTT	=	Dithiothreitol
E2F	=	Elongation factor 2
E6AP	=	E6 associate protein
EDTA	=	Ethylenediaminetetraacetate
EGF	=	Epidermal growth factor
ELISA	=	Enzyme linked immunosorbent assay
EV	=	Epidermodysplasia verruciformis
FDA	=	Food and Drug Administration
GST	=	Glutathione S-transferase
HAuCl ₄	=	Chloroauric acid
HBV	=	Hepatitis B virus
HCl	=	Hydrochloric acid
HEPES	=	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HPV	=	Human papillomavirus
Hr	=	Hour
HSIL	=	High-grade squamous intraepithelial lesion
IgG	=	Immunoglobulin G
IPTG	=	Isopropyl β -D-1-thiogalactopyranoside

LIST OF ABBREVIATIONS (Cont.)

K_2CO_3	=	Potassium carbonate
KCl	=	Potassium chloride
kDa	=	Kilodalton
KH_2HPO_4	=	Dipotassium hydrogen phosphate
L	=	Liter
LSIL	=	Low-grade squamous intraepithelial lesion
M	=	Molar
$MgCl_2$	=	Magnesium chloride
Min	=	Minute
ml	=	Milliliter
mM	=	Millimolar
Na_2HPO_4	=	Sodium phosphate
NaCl	=	Sodium chloride
NCR	=	Non-coding region
nm	=	Nanometer
OD	=	Optical density
ORF	=	Open reading frame
Pap	=	Papanicolaou-stained
PCR	=	Polymerase chain reaction
PDGE	=	Platelet-derived growth factor
PSA	=	Prostate-specific antigen
Rb	=	Retinoblastoma protein
RFLP	=	Restriction fragment length polymorphism
RNA	=	Ribonucleic acid
rpm	=	Revolutions per minute
SB	=	Southern Blot
SDS	=	Sodium dodecylsulfate
SDS-PAGE	=	Sodiumdodecylsulfate polyacrylamide gel electrophoresis
ssDNA	=	Single strand DNA

LIST OF ABBREVIATIONS (Cont.)

TBS	=	Tris buffer saline
TEMED	=	Tetramethylethylenediamine
μ l	=	microliter
URR	=	Upper regulatory region