CHAPTER V

RESULTS

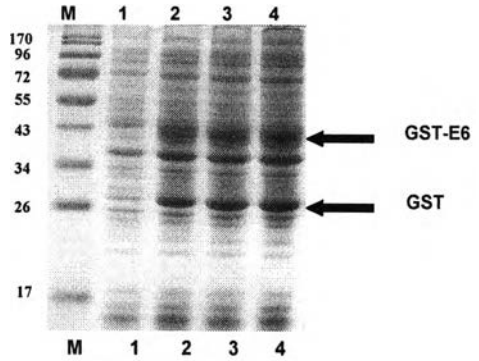
1. Expression of recombinant proteins

To optimize the expression conditions, concentration of IPTG (0.25, 1.0mM), temperature (25 ^{0}C and 37 ^{0}C) and duration of induction (4, 6, 8 hr) were determined as shown in figure 8 and 9. The expression of *E.coli* BL-21(DE3) containing pGEX-3X-MT-E6 induced by IPTG was at 25^{0}C while at 37^{0}C no expression was observed (Fig.8). Moreover, at 25^{0}C the expression was observed both 0.25 and 1.0 mM IPTG induced, but the expression by 0.25 mM IPTG induced at 6 hr was more than 1.0 mM IPTG induced (Fig.8A and B). The expression of *E.coli* BL-21(DE3) containing pGEX-4T-2-L1 induced by IPTG was also done both at 25^{0}C and 37^{0}C, and at 37^{0}C more express than 25^{0}C (Fig.9). The results indicated that optimum expression condition of recombinant L1 protein induced by 1 mM IPTG was at 37 ^{0}C for 4 hr (Fig.9), while recombinant E6 protein was optimally induced with 0.25 mM IPTG at room temperature (25^{0}C) for 6 hr (Fig.8). These conditions were used through out this study for protein expression and SDS-PAGE analysis.

2. Purification of recombinant proteins

After the HPV16 L1 and E6 proteins were expressed under optimal conditions, the GST-HPV16 L1 was found in inclusion bodies associated to the pellet of bacterial cell lysate, while the GST-HPV16E6 was found soluble in cell lysate supernatant (Fig.10). Then the L1 protein in cell pellet was solubilized in 8M urea solution. This 8M urea-soluble protein containing GST-HPV16L1 was analysed by SDS-PAGE showing the fusion protein band at about 80 kDa (Fig.11). The GST-HPV16E6 soluble protein was directly purified by GST affinity chromatography. The eluted protein from the column was further analysed by SDS-PAGE and Western blot as illustrated in figure 12 A and B, respectively. The E6 fusion protein band was about 43 kDa and the GST band was about 26 kDa. Under these conditions of expression and purification, approximately 0.8

mg of GST-HPV16L1 and 2 mg of GST-HPV16 E6 proteins were earned from 2 liters of bacteria culture.



A

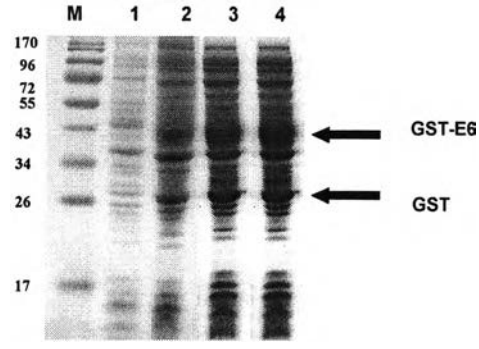
M: Marker

1: Non induced by IPTG

2: Induced by 1.0 mM IPTG, 25 °C, 4 hr.

3: Induced by 1.0 mM IPTG, 25 °C, 6 hr.

4: Induced by 1.0 mM IPTG, 25 °C, 8 hr.



B

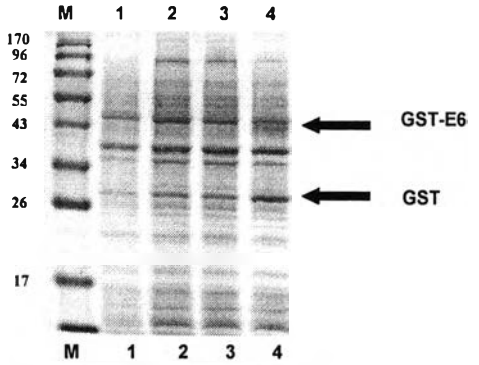
M: Marker

1: Non induced by IPTG

2: Induced by 0.25 mM IPTG, 25 °C, 4 hr.

3: Induced by 0.25 mM IPTG, 25 °C, 6 hr.

4: Induced by 0.25 mM IPTG, 25 °C, 8 hr.



C

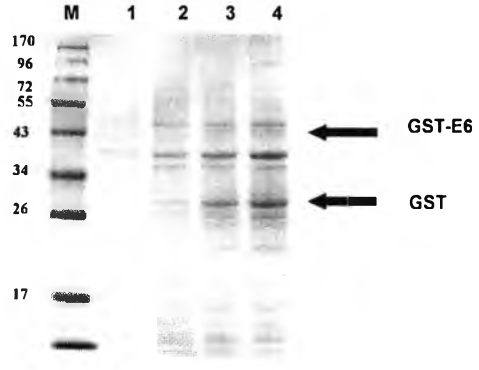
M: Marker

1: Non induced by IPTG

2: Induced by 1.0 mM IPTG, 37 °C, 4 hr.

3: Induced by 1.0 mM IPTG, 37 °C, 6 hr.

4: Induced by 1.0 mM IPTG, 37 °C, 8 hr.



D

M: Marker

1: Non induced by IPTG

2: Induced by 0.25 mM IPTG, 37 °C, 4 hr.

3: Induced by 0.25 mM IPTG, 37 °C, 6 hr.

4: Induced by 0.25 mM IPTG, 37 °C, 8 hr.

Figure8. SDS-PAGE analysis with coomassie blue staining of the GST-HPV16E6 fusion proteins in *E.coli BL-21(DE3)* with various conditions of induction by IPTG: concentration of IPTG, temperature and duration of IPTG induction.

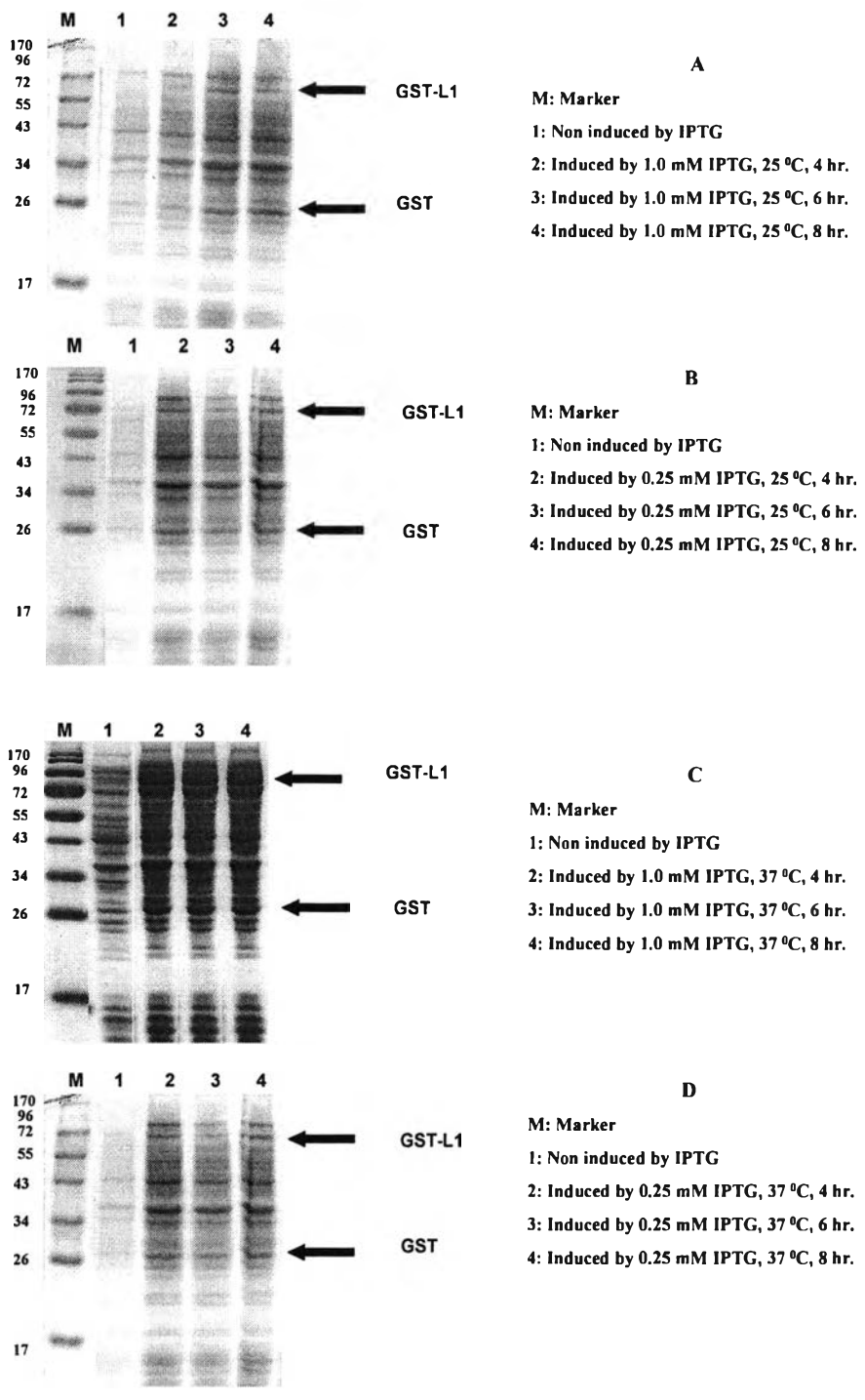


Figure9. SDS-PAGE analysis with coomassie blue staining of the GST-HPV16L1 fusion proteins in *E.coli BL-21(DE3)* with various conditions of induction by IPTG: concentration of IPTG, temperature and duration of IPTG induction.

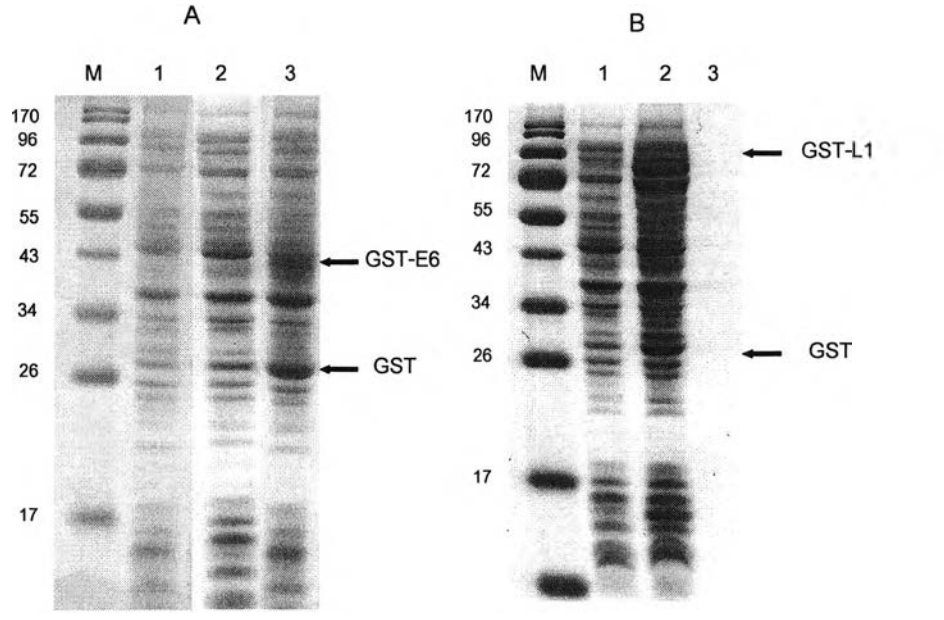


Figure10. SDS-PAGE analysis with coomassie blue staining of expression of fusion proteins. A: GST-HPV16E6, B: GST-HPV16L1 lane M; Marker, lane1; whole cell lysate from *E.coli BL-21(DE3)* containing pGEX-3X-MT-E6 or pGEX-4T-2-L1 non-induce by IPTG, lane2; supernatant of cell lysate from *E.coli BL-21(DE3)* containing pGEX-3X-MT-E6 or pGEX-4T-2-L1 were collected after sonication, lane3; pellet of cell lysate from *E.coli BL-21(DE3)* containing pGEX-3X-MT-E6 or pGEX-4T-2-L1 were collected after sonication.

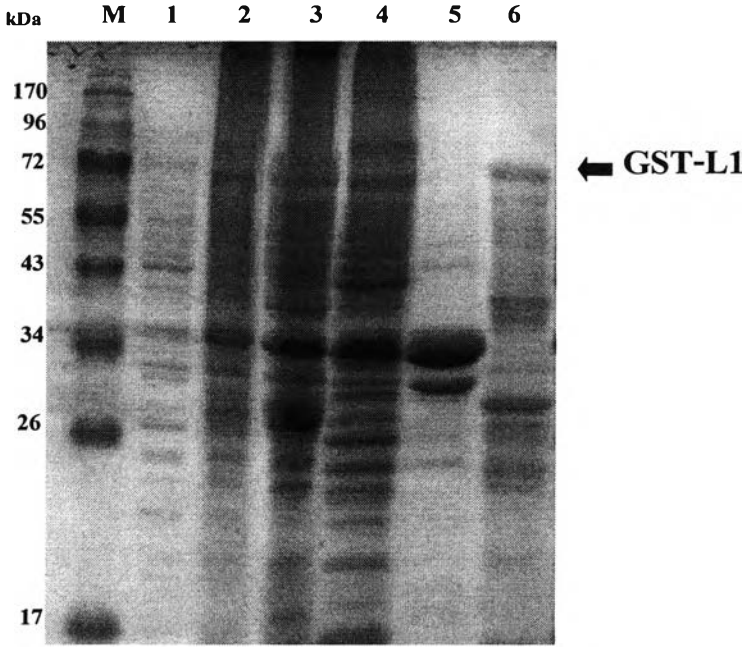


Figure 11. SDS-PAGE analysis of expression and purification of GST-HPV16L1. Lane M; protein marker, lane1 and 2; whole cell lysate from *E.coli BL-21(DE3)* containing pGEX-4T-2-L1 non-induce and induced by IPTG, respectively. Lane3; inclusion bodies form *E.coli BL-21(DE3)*, lane4; soluble fraction from *E.coli BL-21(DE3)*, lane5; flow-through washed *E.coli BL-21(DE3)*, lane6; purified GST-HPV16L1 in urea solution.

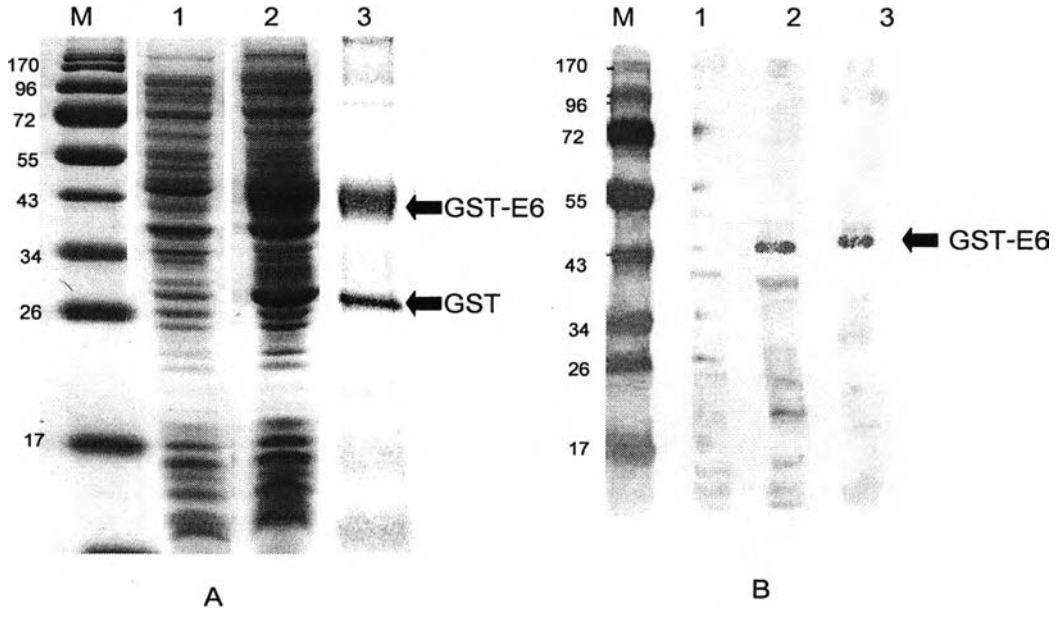


Figure 12. SDS-PAGE and Western blot analysis of expression and purification of GST-HPV16E6. A: SDS-PAGE stained with coomassie blue and B: Western blotting, the purified GST-E6 was detected with mouse anti-E6 monoclonal antibody, lane1; whole cell lysate from *E.coli BL-21(DE3)* non-induced by IPTG, lane2; whole cell lysate from *E.coli BL-21(DE3)* containing pGEX-3X-MT-E6 induced by IPTG, and lane3; purified of GST- HPV16E6.

3. Optimization of goldnanoparticles and antibody condition

Standard curves, adsorption isotherms, were determined for each antibody in order to determine the minimum amount of antibody and optimum pH conditions required for stabilization of goldnanoparticles. The pH of AuNPs, i.e., pH4, 5, 6, 7, 8 and 9 were adjusted before conjugating with diluted antibody (1:200, 1:400, 1:600, 1:800 and 1:1000). These pH values were determined from adsorption isoterms for each proteins and depended on both variations in the isoelectric points of the molecules and on the molecule fraction of each protein. The optical densities (O.D) at 580 nm were plotted against pH to determine the pH range where stabilization of AuNPs by the antibody were seen in figure 13-15. A curve was generated from series of readings and the point where there curve first appears non-linear curve with the x axis was taken as the minimum quantity of antibody needed to stabilize the AuNPs. The pH of solution of AuNPs for conjugating were adjusted at pH range 4-9 for HPV-16L1 antibody (Fig.13), pH 5-8 for HPV-16E6 antibody (Fig.14) and pH8 for normal goat antibody (Fig.15). The results showed optimum pH of solution of AuNPs for conjugating to all antibodies are pH8. For minimum quantity of antibody needed to stabilized the AuNPs was follows: final dilution 1:1000 for HPV-16L1 antibody (Fig.13); final dilution 1:1000 for HPV-16E6 antibody (Fig.14) and final dilution 1:400 for normal goat antibody (Fig.15). The results described above showed the minimum concentration of all antibodies for stabilized of AuNPs as a final dilution 1:400.

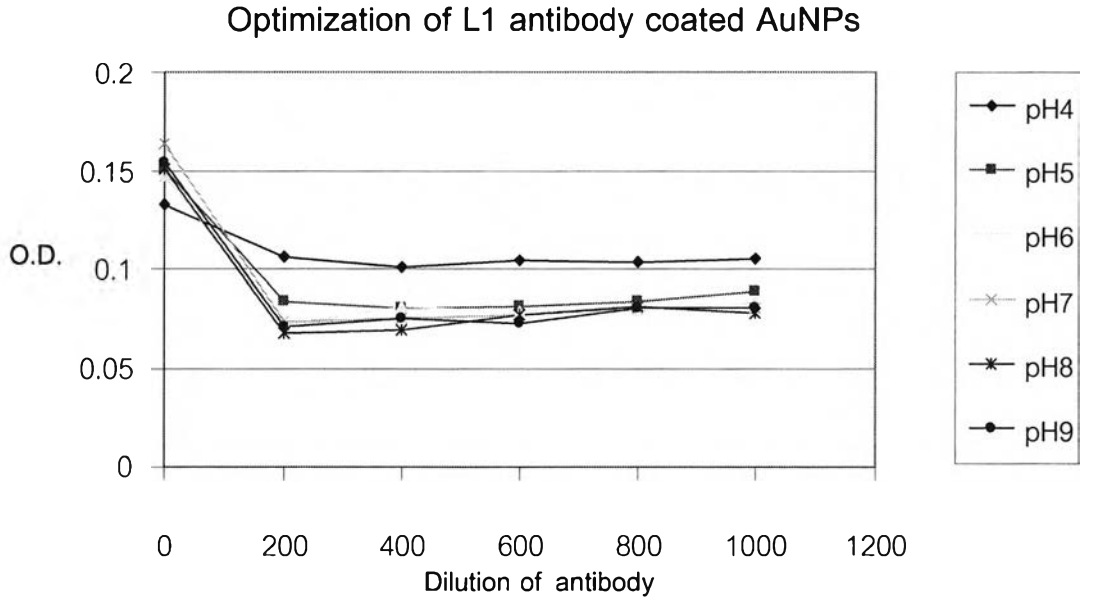


Figure13. Optimization of HPV-16L1 antibody coated with AuNPs. All AuNPs was optimized by varying pH of colloidal gold solution (pH4, 5, 6, 7, 8, and 9). HPV-16L1 goat polyclonal antibody was optimized by varying dilution concentration of antibody (final dilution 200, 400, 600, 800 and 1000). The optical densities were measured at 580 nm after 10% NaCl were added to each tube of solution, mixed rapidly and allowed to stand for 5 min.

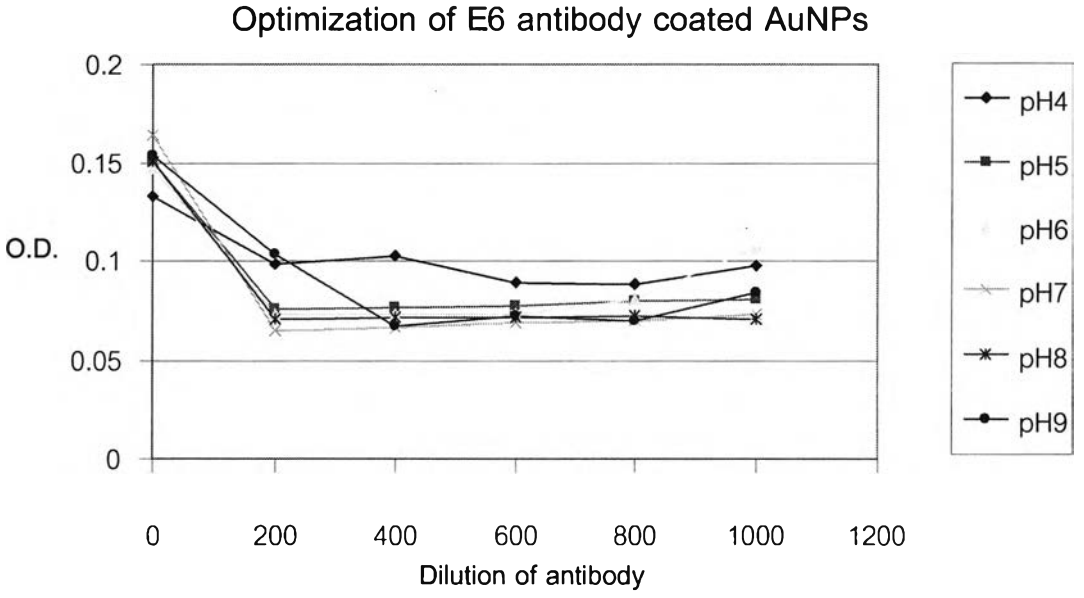


Figure14. Optimization of HPV-16E6 antibody coated with AuNPs. All AuNPs was optimized by varying pH of colloidal gold solution (pH4, 5, 6, 7, 8, and 9). HPV-16E6 goat polyclonal antibody was optimized by varying dilution concentration of antibody (final dilution 200, 400, 600, 800 and 1000). The optical densities were measured at 580 nm after 10% NaCl were added to each tube of solution, mixed rapidly and allowed to stand for 5 min.

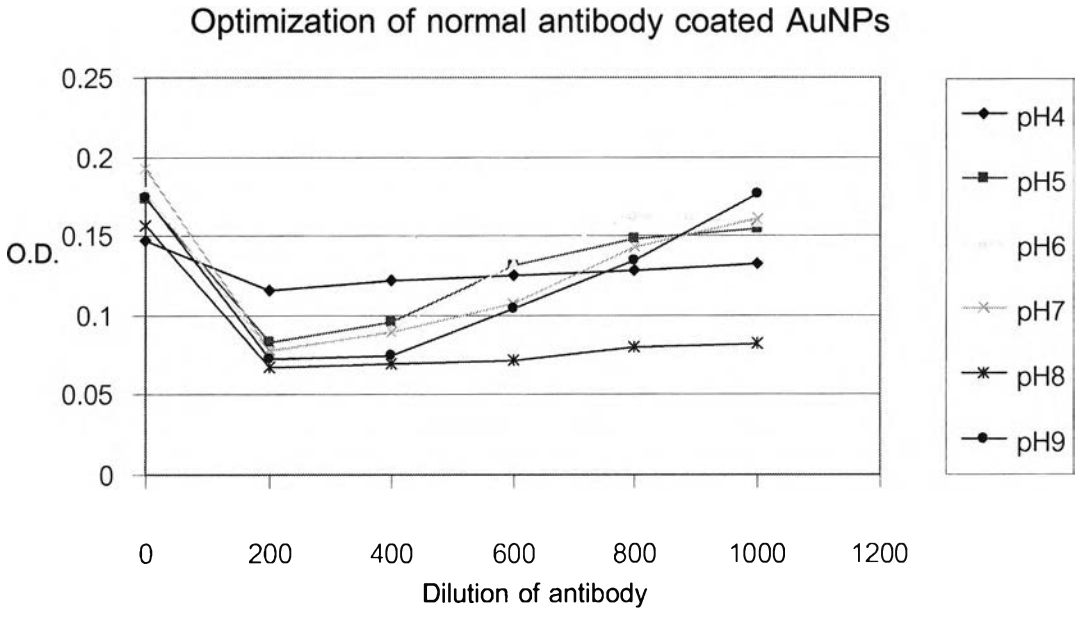


Figure15. Optimization of normal goat antibody coated with AuNPs. All AuNPs was optimized by varying pH of colloidal gold solution (pH4, 5, 6, 7, 8, and 9). Normal goat polyclonal antibody was optimized by varying dilution concentration of antibody (final dilution 200, 400, 600, 800 and 1000). The optical densities were measured at 580 nm after 10% NaCl were added to each tube of solution, mixed rapidly and allowed to stand for 5 min.

4. Immunogoldagglutination assay with HPV-16 fusion proteins

The immunogoldagglutination of HPV-16L1 or HPV-16E6 coated AuNPs in the presence of protein L1 or E6 takes several hours to complete. The agglutinate after 24 hours of immunogoldagglutination were shown in figure 16. However, the absorbtion change at 620 nm of the immunogoldagglutination were measured within 120 min. A typical spectrum of AuNPs solution showing a plasmon resonance peak at 520 nm. The agglutination of AuNPs leads to increase in absorbance at longer wavelength (600-750) which, in this study used absorbance at 620 nm. Effect of temperature of immunogoldagglutination on the rate of agglutination were showed in figure 17-19. The rate of agglutinate of HPV-16L1 coated AuNPs reacted with 40 µg L1 fusion proteins and HPV-16E6 coated AuNPs reacted with 40 µg E6 fusion proteins increase at room temperature (Fig.17 and 18 respectively). Moreover, in these experiments indicated increasing temperature to 37^0C increase the rate of nonspecific agglutination (Fig.19) while decreasing the temperature to 4^0C the agglutination did not occur.

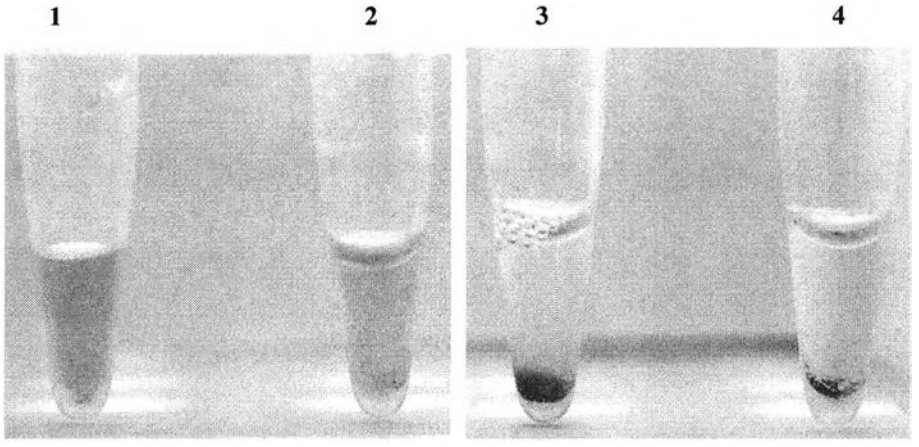


Figure16. Immunogoldagglutination assay with HPV antigens. The agglutination observed after 24 hr. of immunogoldaggutination assay at room temperature. Tube 1 and 2 were the reaction between normal goat antibody coated AuNPs and 40μg of L1 and E6 fusion proteins respectively. Tube3 was the reaction between HPV-16E6 antibody coated AuNPs and 40μg of E6 fusion proteins. Tube4 was the reaction between HPV-16L1 antibody coated AuNPs and 40μg of L1 fusion proteins.

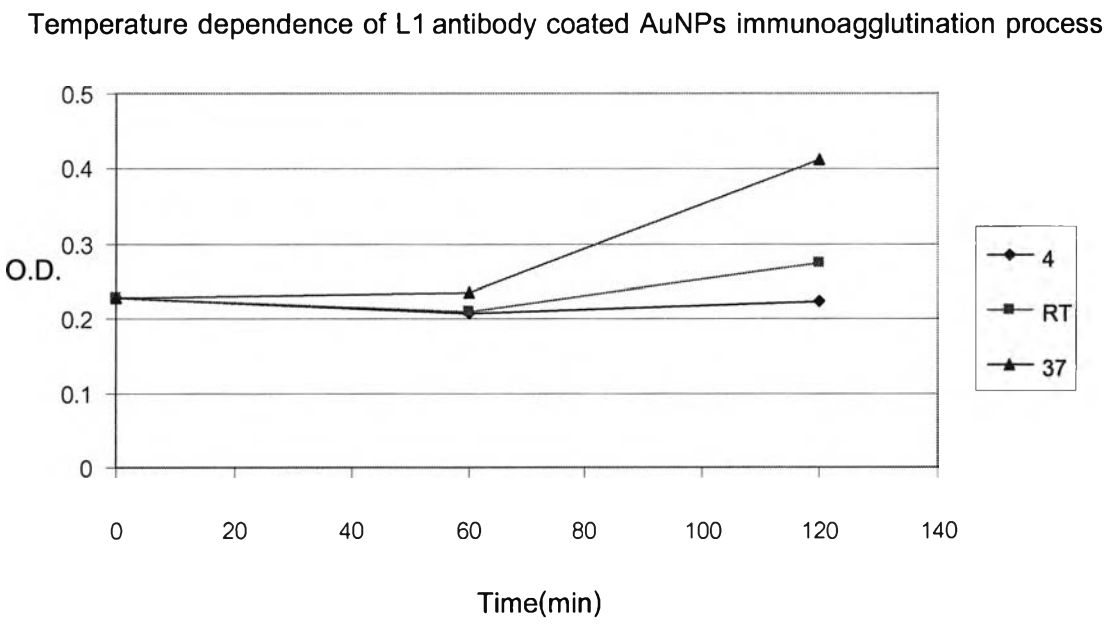


Figure17. Temperature dependence of HPV-16L1 coated AuNPs immunogoldagglutination process. The assay was performed using HPV-16L1 antibody coated AuNPs and 40μg of L1 fusion proteins and monitored for 2 hr. The absorption change at 620 nm was measured at 4^0C, room temperature(RT) and 37^0C.

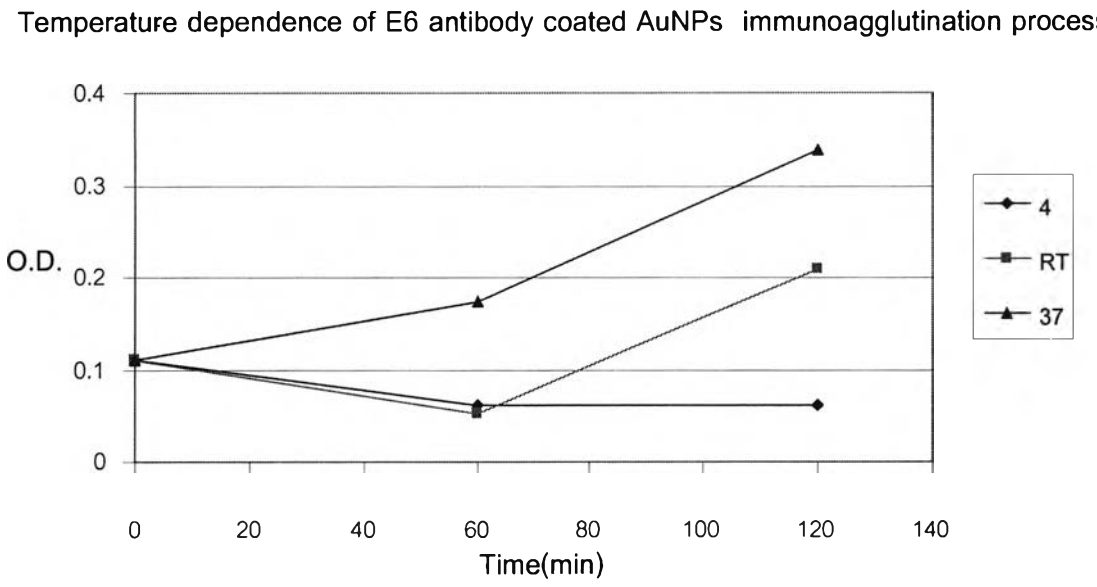


Figure18. Temperature dependence of HPV-16E6 coated AuNPs immunogoldagglutination process. The assay was performed using HPV-16E6 antibody coated AuNPs and 40μg of E6 fusion proteins and monitored for 2 hr. The absorption change at 620 nm was measured at 4^0C, room temperature(RT) and 37^0C.

Temperature dependence of nomal antibody coated AuNPs immunoagglutination process

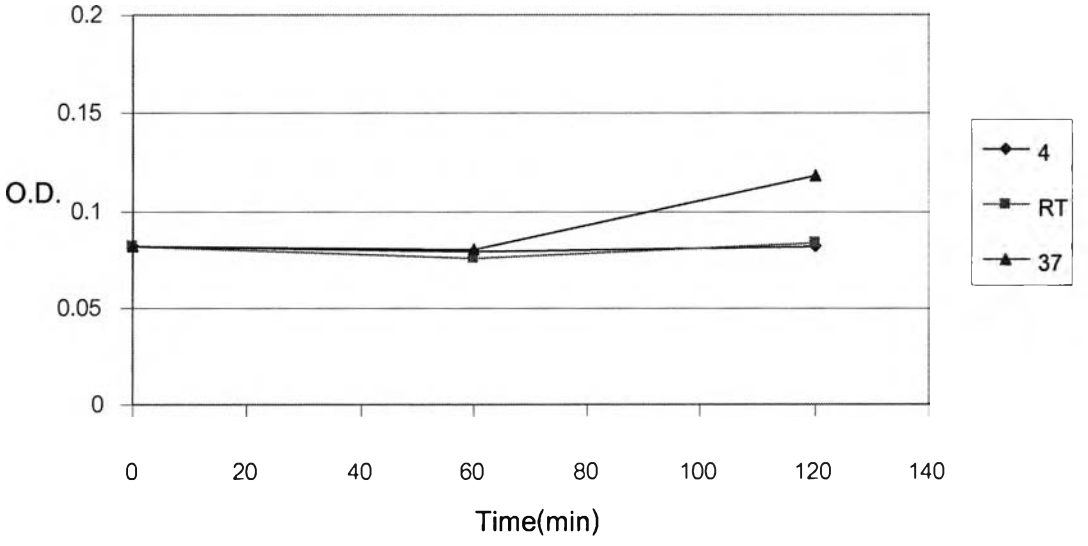


Figure19. Temperature dependence of normal goat antibody coated AuNPs immunogoldagglutination process. The assay was performed using normal goat antibody coated AuNPs and 40μg of L1 fusion proteins and monitored for 2 hr. The absorption change at 620 nm was measured at 4^0C, room temperature(RT) and 37^0C.

5. Sensitivity of immunogoldagglutination assay with HPV-16 fusion proteins

The minimum amount of each HPV-16L1 or E6 fusion proteins necessary to detection by immunogoldagglutination assay was determined by measuring an absorption change at 620 nm using four-fold dilution (0.625, 2.5, 10 and 40 μg) of L1 or E6 fusion proteins reacted with HPV-16L1 or E6 coated AuNPs. An absorption change at 620 nm of calibration curve for HPV-16L1 or E6 fusion proteins are shown in figure 20 and 21, respectively. The limit of detection of HPV-16L1 or E6 fusion protein in the assay was 2.5 μg. Moreover at higher concentration of HPV-16L1 fusion proteins, the agglutination process was inhibited, which may be due to the blocking of active sites.

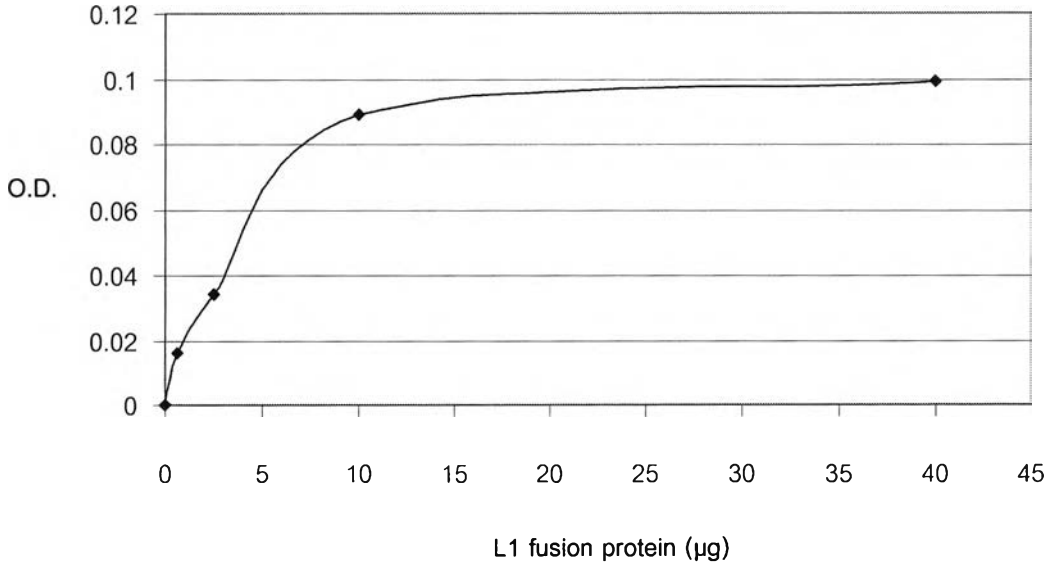


Figure20. Calibration curve describing the absorption change at 620 nm against HPV-16L1 fusion proteins. A dynamic range of two orders of magnitude in concentration was observed.

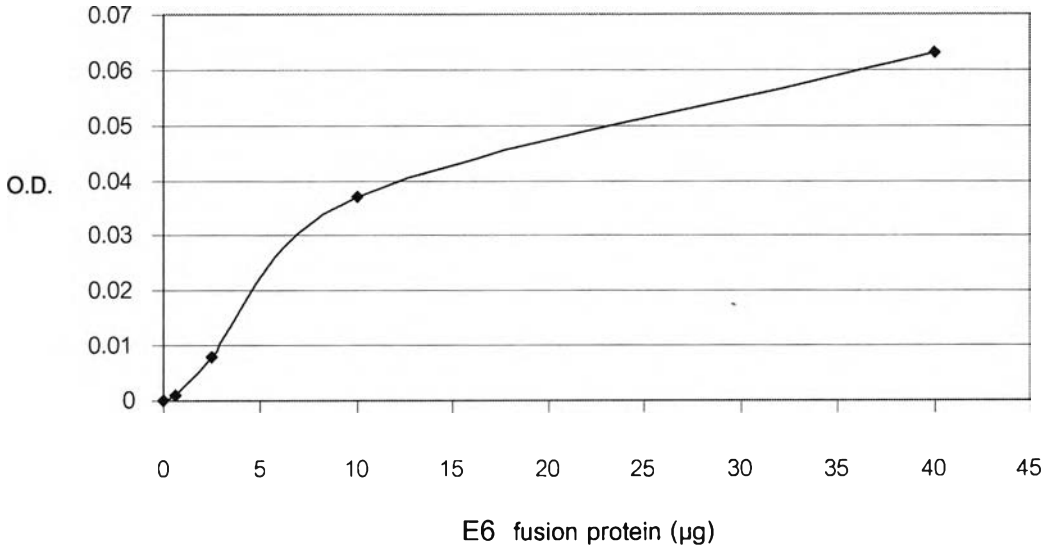


Figure21. Calibration curve describing the absorption change at 620 nm against HPV-16E6 fusion proteins. A dynamic range of two orders of magnitude in concentration was observed.

6. Control experiments of immunogoldagglutination assay with HPV-16 fusion proteins

Several control experiments were carried out to validate selectivity of the immunogoldagglutination assay. The results of these experiments were shown in figure 22 and 23. Curve 1 showed the absorption intensity at 620 nm of solution containing HPV-16 monoclonal antibody coated AuNPs and HPV-16 fusion protein. Curve 2 showed the absorption intensity at 620 nm of solution containing HPV-16 polyclonal antibody coated AuNPs and HPV-16 fusion protein. Curve 3 showed the absorption intensity at 620 nm of solution containing normal goat antibody coated AuNPs and HPV-16 fusion protein.Curve 4 showed the absorption intensity at 620 nm of uncoated AuNPs solution at pH 8. Curve 5 showed the absorption intensity at 620 nm of a solution antibody coated AuNPs in the absence of HPV-16 fusion proteins. The absorption intensity at 620 nm remained constant for 2 hours in these control experiments. Curve 2 showed a positive response of the assay, while another curve no absorption change was observed for 2 hr. In curve 1 there was no response because this was attributed to the lack of multiple binding sites and the inability of monoclonal antibodies to form agglutinates among a large numbers of particle. And curve 3, no absorption change was observed, indicating that no nonspecific agglutination and no cross-reactivity of the antigen occurred.

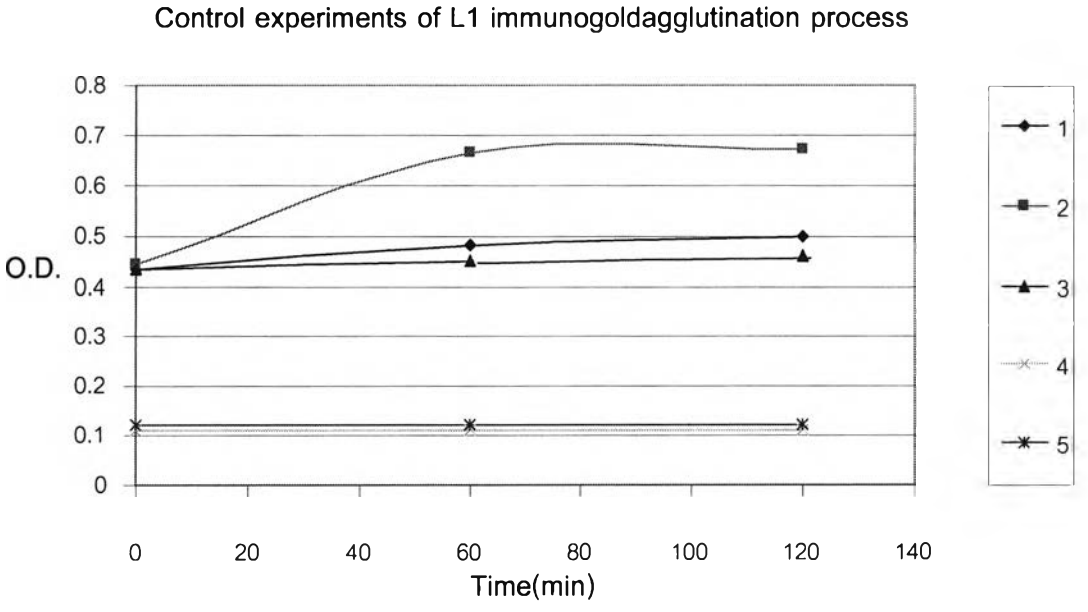


Figure22. Control experiments of HPV-16L1 immunogoldagglitination assay. Curve1: solution containing HPV-16L1 monoclonal antibody coated AuNPs and HPV-16L1 fusion protein. Curve 2: solution containing HPV-16L1 polyclonal antibody coated AuNPs and HPV-16L1 fusion protein. Curve 3: solution containing normal goat antibody coated AuNPs and HPV-16L1 fusion protein. Curve 4: uncoated AuNPs solution at pH 8. Curve 5: solution of HPV-16L1polyclonal antibody coated AuNPs in the absence of HPV-16 fusion proteins. All experiments were measured optical densities change at 620 nm for 2 hours.

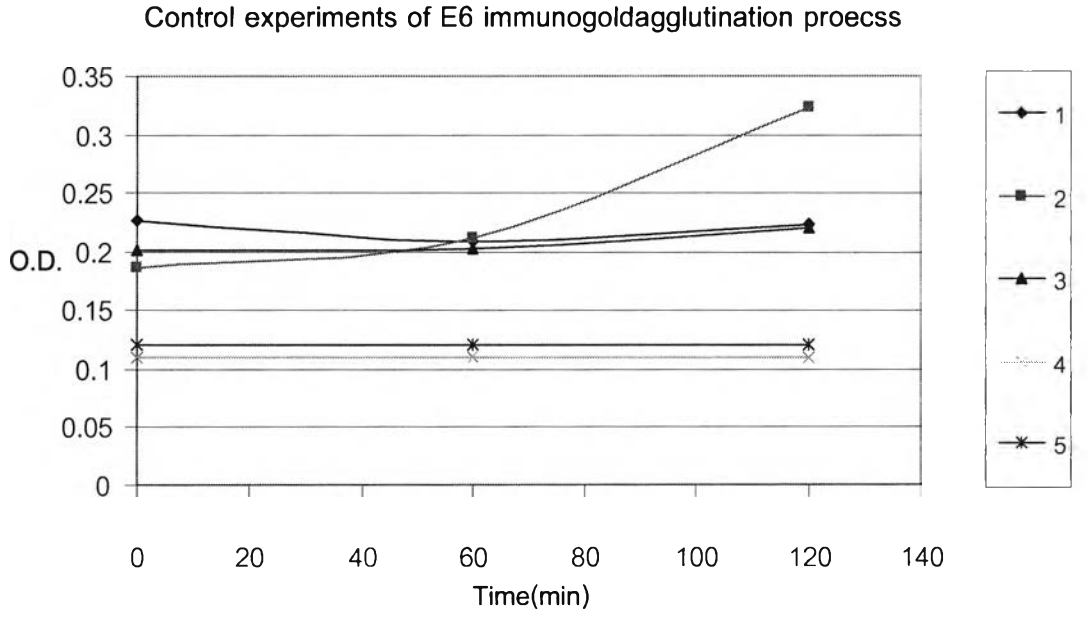


Figure23. Control experiments of HPV-16E6 immunogoldagglitination assay. Curve1: solution containing HPV-16E6 monoclonal antibody coated AuNPs and HPV-16 E6 fusion protein. Curve 2: solution containing HPV-16 E6 polyclonal antibody coated AuNPs and HPV-16 E6 fusion protein. Curve 3: solution containing normal goat antibody coated AuNPs and HPV-16 E6 fusion protein. Curve 4: uncoated AuNPs solution at pH 8. Curve 5: solution of HPV-16 E6 polyclonal antibody coated AuNPs in the absence of HPV-16 fusion proteins. All experiments were measured optical densities change at 620 nm for 2 hours.

7. Immunogoldagglutination assay with control cell line

To confirm the practicability of this assay for clinical testing we carried out a series of experiments consisting of protein extraction from human cervical cell line, CaSki cell line. The CaSki cell line is a human cervical carcinoma cell line with about 600 copies of HPV-16. The agglutination of this assay was found in reaction between HPV-16E6 antibody coated AuNPs and CaSki cell lysate, but not found in reaction between HPV-16L1 antibody coated AuNPs and CaSki cell lysate after 24 hours of these assays (Fig.24).

8. Sensitivity of immunogoldagglutination assay with control cell line

The sensitivity of immunogoldagglutination assay was determined by agglutination of HPV-16E6 antibody coated AuNPs and E6 proteins from CaSki cell lysate. The CaSki cell lysate from 10^4, $5x10^4$, 10^5, $5x10^5$, 10^6, and $2x10^6$ cell were used for these assays. We found at least $5X10^5$ of CaSki cell lysate could be agglutinated with HPV-16E6 antibody coated AuNPs within 30 min (Table10).

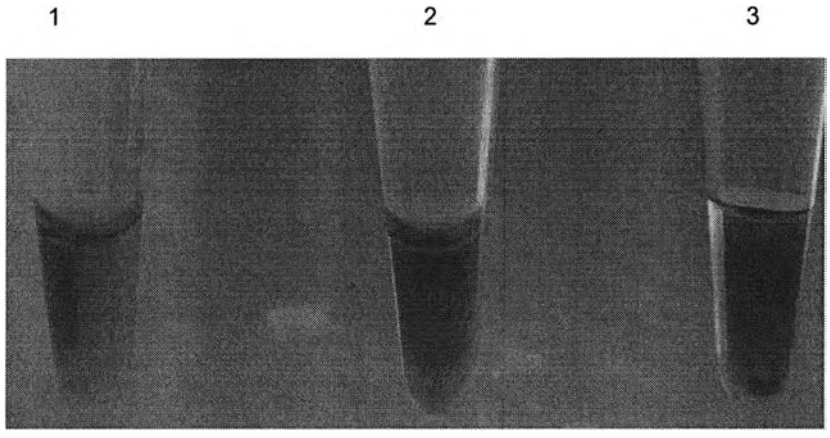


Figure24. Immunogoldagglutination assay with control cell line. The agglutination observed after 24 hr. of immunogoldaggutination assay at room temperature. Tube 1 was the reaction between normal goat antibody coated AuNPs and 10^6 of CaSki cell lysate. Tube 2 was the reaction between HPV-16L1 antibody coated AuNPs and 10^6 of CaSki cell lysate. Tube3 was the reaction between HPV-16E6 antibody coated AuNPs and 10^6 of CaSki cell lysate.

Table10. Immunogoldagglutination assay of HPV-16E6 antibody coated AuNPs with CaSki cell lysate.

Number of CaSki cell	Time (hr.)											
	0.5	1	2	3	4	5	6	7	8	16	20	24
2,000,000	-	-	-	-	-	W	W	W	W	+	+	+
1,000,000	W	W	W	W	W	W	+	+	+	+	+	+
500,000	W	W	W	W	W	W	+	+	+	+	+	+
100,000	-	W	W	W	W	W	W	+	+	+	+	+
50,000	-	-	-	-	-	-	-	-	-	-	-	-
10,000	-	-	-	-	-	-	-	-	-	-	-	-

- : No agglutination

\+ : Agglutination

W : Weakly agglutination

9. Specificity of immunogoldagglutination assay

The specificity of immunogoldagglutination assay based on the results of agglutination are also shown in table 11 and 12. For different human tumor cell lysate containing other HPV types, i.e., Hela cell line containing HPV type18, ME-180 containing HPV type 18 and 39, MS751 containing HPV type 18 and 45, and C-33A cell line does not have HPV DNA, no agglutination was observed, indicating that no nonspecific agglutination (Table 11). And other organisms, some bacteria strains (*S.aureus, S.viidans, Enterococcus* spp., *E.coli* and *Ps.aeruginasa*) which are commonly found in vagina, no agglutination was observed, indicating no cross-reactivity of the bacterial antigens was found (Table 12).

Table11. Immunoagglutination assays of HPV-16E6 antibody coated AuNPs with control cell lysates.

Type of cell lysate (HPV type)	Time (hr.)											
	0.5	1	2	3	4	5	6	7	8	16	20	24
CaSki (16)	W	W	W	W	W	W	+	+	+	+	+	+
SiHa (16)	-	-	-	-	-	-	-	-	-	-	-	w
Hela (18)	-	-	-	-	-	-	-	-	-	-	-	-
ME-180 (18,39)	-	-	-	-	-	-	-	-	-	-	-	-
MS751 (18,45)	-	-	-	-	-	-	-	-	-	-	-	-
C-33A	-	-	-	-	-	-	-	-	-	-	-	-
Hep-2	-	-	-	-	-	-	-	-	-	-	-	-

- : No agglutination

\+ : Agglutination

W : Weakly agglutination

Table 12. Immunoagglutination assays of HPV-16E6 or L1 antibody coated AuNPs with bacteria cell lysates.

Type of cell lysate	Time (hr.)											
	0.5	1	2	3	4	5	6	7	8	16	20	24
CaSki	W	W	W	W	W	W	+	+	+	+	+	+
S.aureus	-	-	-	-	-	-	-	-	-	-	-	-
Viridans strep.	-	-	-	-	-	-	-	-	-	-	-	-
Enterococcus spp.	-	-	-	-	-	-	-	-	-	-	-	-
E.coli	-	-	-	-	-	-	-	-	-	-	-	-
Ps.aeruginosa	-	-	-	-	-	-	-	-	-	-	-	-

10. HPV detection and typing in clinical samples

HPV detection in control cell lines and clinical samples were performed by PCR amplification of HPV L1 region. The system used L1C1-L1C2 primer set produced approximately 250 bp amplified product. As a control for DNA amplification, human ß-globin gene was amplified by GH20 and PC04 primers and produced 268 bp amplified product. After that to amplification of HPV type 16 detection using type specific Pr1/Pr2 primer set and produced 119 bp amplified product (Fig.25). Forty of patients were 100% (40/40) of HPV DNA detection (Table13). The prevalence of HPV type 16 infection in normal pathology patients was 10% (1/10), LSIL (CIN1) group was 70% (7/10), HSIL (CIN2 and CIN3) group was 100% (10/10) and CaCx group was 70% (7/10) (Table13).

11. Immunogoldagglutination in clinical samples

In clinical samples, 40 cervical swab samples of confirmed pathology (normal, LSIL, HSIL and CaCx) were tested with immuogoldagglutination assay. The results were shown in table14. All 16 samples HPV-16 DNA-negative were negative for these assays. In HPV-16 samples, 1 sample pathology-negative was positive for L1 protein, while were negative for E6. In LSIL(CIN1) group 1 out of 7 (14%) was positive for L1 protein, while all 7 samples were negative for E6. In HSIL group, all 4 CIN2 negative for E6 protein, 3 out of 4 CIN2(75%) were positive for L1, 4 out of 6 CIN3 (67%) were positive for E6 protein and 5 out of 6 CIN3 (83%) were positive for L1 protein. CaCx patients associated with HPV-16 were found 5 out of 7 (71%) were positive for both E6 and L1 protein. Conclusion of these results were shown in table 15.

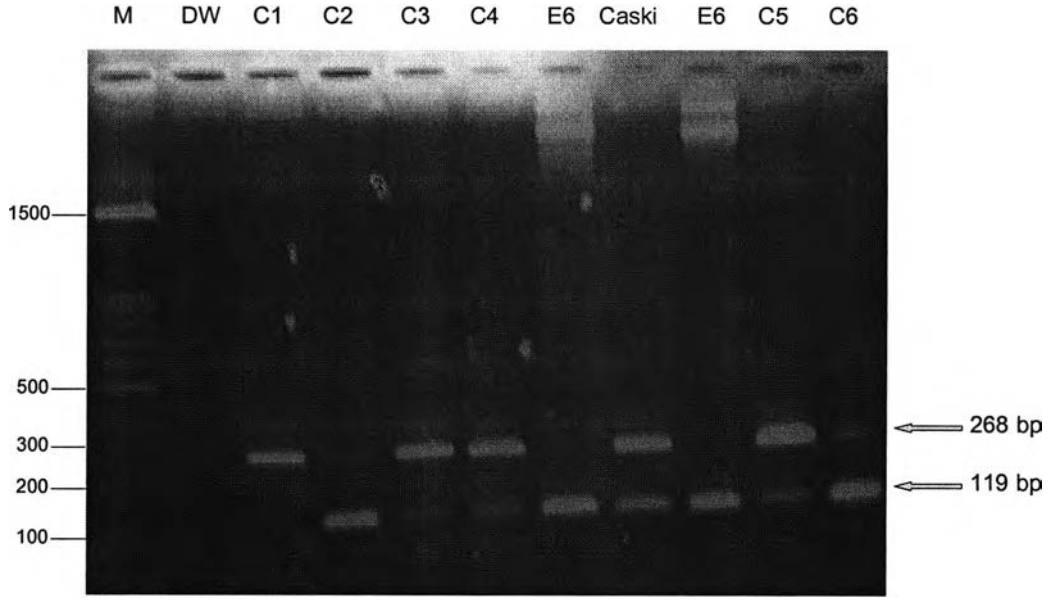


Figure 25. Amplification of HPV-16 E6 gene detection using type specific Pr1/Pr2 primer set and GH20/PC04 primers set. The E6 gene could be amplified from clinical samples and produced 119 bp and a control for DNA amplification, human ß-globin gene produced 268 bp. M: 100 bp DNA ladder, E6: pCIneo E6 plasmid as positive control, Caski: control cell line which containing HPV-16, C1-C6: cervical swab from CaCx patients.

Table13. HPV detection and typing in clinical samples

Samples	Pathology	HPV DNA	HPV-16 DNA
N1	Normal	+	-
N2	Normal	+	-
N3	Normal	+	-
N4	Normal	+	-
N5	Normal	+	-
N6	Normal	+	-
N7	Normal	+	-
N8	Normal	+	-
N9	Normal	+	-
N10	Normal	+	+
L1	CIN1	+	+
L2	CIN1	+	+
L3	CIN1	+	+
L4	CIN1	+	+
L5	CIN1	+	+
L6	CIN1	+	+
L7	CIN1	+	+
L8	CIN1	+	-
L9	CIN1	+	-
L10	CIN1	+	-
H1	CIN2	+	+
H2	CIN2	+	+
H3	CIN2	+	+
H4	CIN2	+	+
H6	CIN3	+	+
H7	CIN3	+	+
H8	CIN3	+	+
H9	CIN3	+	+
H10	CIN3	+	+
C1	CaCx	+	-
C2	CaCx	+	+
C3	CaCx	+	+
C4	CaCx	+	+
C5	CaCx	+	+
C6	CaCx	+	+
C7	CaCx	+	+
C8	CaCx	+	-
C9	CaCx	+	-
C10	CaCx	+	+

*N: Normal pathology group, L: LSIL group, H: HSIL group, C: Cervical cancer group

Table14. Immunoagglutination assays in clinical samples

Samples	Pathology	HPV-DNA	HPV-16 DNA	HPV-16 detection (Immunoagglutination assays)	
				L1 detection	E6 detection
N1	Normal	+	-	-	-
N2	Normal	+	-	-	-
N3	Normal	+	-	-	-
N4	Normal	+	-	-	-
N5	Normal	+	-	-	-
N6	Normal	+	-	-	-
N7	Normal	+	-	-	-
N8	Normal	+	-	-	-
N9	Normal	+	-	-	-
N10	Normal	+	+	+	-

*+: Positive

-: Negative

Table14. Immunoagglutination assays in clinical samples (continue)

Samples	Pathology	HPV-DNA	HPV-16 DNA	HPV-16 detection (Immunoagglutination assays)	
				L1 detection	E6 detection
L1	CIN1	+	+	-	-
L2	CIN1	+	+	-	-
L3	CIN1	+	+	-	-
L4	CIN1	+	+	-	-
L5	CIN1	+	+	-	-
L6	CIN1	+	+	-	-
L7	CIN1	+	+	+	-
L8	CIN1	+	-	-	-
L9	CIN1	+	-	-	-
L10	CIN1	+	-	-	-

*+: Positive

-: Negative

Table14. Immunoagglutination assays in clinical samples (continue)

Samples	Pathology	HPV-DNA	HPV-16 DNA	HPV-16 detection (Immunoagglutination assays)	
				L1 detection	E6 detection
H1	CIN2	+	+	-	-
H2	CIN2	+	+	+	-
H3	CIN2	+	+	+	-
H4	CIN2	+	+	+	-
H5	CIN3	+	+	-	-
H6	CIN3	+	+	+	+
H7	CIN3	+	+	+	-
H8	CIN3	+	+	+	+
H9	CIN3	+	+	+	+
H10	CIN3	+	+	+	+

*+: Positive

-: Negative

Table14. Immunoagglutination assays in clinical samples (continue)

Samples	Pathology	HPV-DNA	HPV-16 DNA	HPV-16 detection (Immunoagglutination assays)	
				L1 detection	E6 detection
C1	CaCx	+	-	-	-
C2	CaCx	+	+	+	+
C3	CaCx	+	+	+	+
C4	CaCx	+	+	-	-
C5	CaCx	+	+	+	+
C6	CaCx	+	+	-	-
C7	CaCx	+	+	+	+
C8	CaCx	+	-	-	-
C9	CaCx	+	-	-	-
C10	CaCx	+	+	+	+

*+: Positive

-: Negative

Table15. Comparison of HPV-16 detection from clinical samples by HPV-16 PCR versus immunogoldagglutination assay.

Pathology	HPV-16 by PCR		HPV-16 by E6 detection (immunogoldagglutination assay)		HPV-16 by L1 detection (immunogoldagglutination assay	
	Results	Number of samples	Results	Number of samples	Results	Number of samples
Normal	Negative	9	Negative	10	Negative	9
	Positive	1	Positive	0	Positive	1
CIN1	Negative	3	Negative	10	Negative	9
	Positive	7	Positive	0	Positive	1
CIN2	Negative	0	Negative	4	Negative	1
	Positive	4	Positive	0	Positive	3
CIN3	Negative	0	Negative	2	Negative	1
	Positive	6	Positive	4	Positive	5
CaCX	Negative	3	Negative	5	Negative	5
	Positive	7	Positive	5	Positive	5