Archives of Razi Institute, Vol. 75, No. 2 (2020) 187-195


DOI: 10.22092/ARI.2018.121095.1205

*Original Article*

# Comparative Evaluation of Human Papillomavirus Type 16 L1 Protein Expressed in Plasmid- and Baculovirus-Based Systems in Insect Cells

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Received 14 August 2018; Accepted 20 October 2018
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**ABSTRACT**

Human papillomavirus (HPV) has been associated with specific types of papillomas, lesions at particular anatomic sites, and malignancies. The HPV16 and HPV18 have been shown to play a role in a variety of carcinomas. The most documented HPV-associated cancer is cervical carcinoma. Suitable antigens are needed to be identified for the diagnostic tests and vaccines and the expression of L1 recombinant protein should be accelerated in papillomaviruses. Therefore, in this study, the expression of the L1 protein of HPV16 was evaluated and compared in insect cells using a plasmid and a baculovirus system. The expression of the L1 protein of HPV16 in insect cells was investigated using a plasmid (InsectDirect) and a baculovirus system (BacMagic). The expressed recombinant proteins were purified from the Sf9 lysate using Ni-NTA resin columns. The characterization of recombinant L1 protein expressed in both systems (BacMagic and InsectDirect) was performed using immunofluorescence, SDS-PAGE, western blot, and dot blot. The yields of the purified proteins from the plasmid- and baculovirus-based systems (10 ml culture; $10^7$ cells) had the ranges of 455-495 µg/ml and 1.44-1.6 mg/ml as analyzed by spectrophotometer, respectively. The SDS-PAGE analysis of purified proteins revealed that the recombinant proteins with the expected size of 58 KDa were produced in both InsectDirect and baculovirus systems. A high degree (95%) of purification was achieved using this system as observed in SDS-PAGE. The purified L1 protein in the baculovirus system was clearly more efficient than the InsectDirect system. The results of this study indicate that the BacMagic system is an appropriate tool for large scale protein production and provides an alternative to the traditional baculovirus system. In addition, the InsectDirect system might provide a rapid and dependable pointer of whether a protein can be successfully produced in a baculovirus system. Both InsectDirect and BacMagic systems present remarkable savings in cost and time.

**Keywords:** Baculovirus, Cloning, Expression, Papillomavirus

**Évaluation Comparative de la Protéine de Type 16 L1 du Papillomavirus Humain Exprimée dans des Systèmes à Base de Plasmides et de Baculovirus dans des Cellules d'Insectes**

**Résumé:** Le papillomavirus humain a été associé aux types spécifiques de papillomes, aux lésions sur des sites anatomiques particuliers et aux tumeurs malignes. Les HPV16 et HPV18 ont un rôle causal dans une variété de carcinomes. Le carcinome du col utérin est le cancer le mieux documenté associé au HPV. Dans cette étude, l'expression de papillomavirus de type 16 L1 dans des cellules d'insectes à l'aide d'un plasmide et d'un système baculovirus a été évaluée et comparée afin de fournir des antigènes appropriés pour les tests de diagnostic et les vaccins ainsi que pour 'accélérer l'expression de la protéine recombinante L1 des papillomavirus. L'expression de la protéine de type 16 L1 du papillomavirus dans des cellules d'insectes a été étudiée en utilisant un plasmide (InsectDirect) et un système baculovirus (BacMagic). Les protéines recombinantes exprimées ont été purifiées à partir du lysat Sf9 en utilisant des colonnes de résine Ni-NTA. La caractérisation de la protéine L1 recombinante exprimée dans les deux systèmes (BacMagic et InsectDirect) a été réalisée par immunofluorescence,

SDS-PAGE, Western blot et dot blot. Les rendements des protéines purifiées analysés par spectrophotométrie dans le système plasmidique (culture de 10 ml; 107 cellules) allait de 455 µg / ml à 495 µg / ml, alors que le système à base de baculovirus (culture de 10 ml; 107 cellules) produisait 1,44 mg / ml à 1,60 mg / ml de protéines recombinantes. Une analyse SDS-PAGE des protéines purifiées a révélé que les protéines recombinantes de taille attendue (58 KDa) étaient produites dans les systèmes InsectDirect et baculovirus. Un degré élevé de purification (95%) a été atteint en utilisant ce système, comme observé dans le profil SDS-PAGE. La protéine L1 purifiée dans le système baculovirus était clairement plus efficace que le système InsectDirect. Les résultats de cette étude indiquent que le système BacMagic est un système approprié pour la production de protéines à grande échelle et constitue une alternative au système baculovirus traditionnel. Le système InsectDirect peut également fournir un indicateur rapide et fiable afin d'évaluer si une protéine peut être produite avec succès dans un système à baculovirus. Les systèmes InsectDirect et BacMagic présentent tous deux des gains de temps et une économie de frais remarquable.

**Mots-clés**: le Baculovirus; le Papillomavirus; l'Expression; le Clonage

## INTRODUCTION

Human papillomaviruses (HPVs) are small, non-enveloped DNA viruses that infect cutaneous, genital, and respiratory epithelia. Particular HPV types have been associated with some specific types of papillomas, lesions at particular anatomic sites, and malignancies. Moreover, HPV is thought to play an etiologic role in other cancers in immunosuppressed individuals. Serological assays are of importance in the diagnosis of viral infections and in seroepidemiological studies in terms of determining the level of immunity to infection. HPVs cannot be propagated in conventional cell cultures or have a poor yield in these cultures. The representation of the major antigenic determinants of HPVs, known as L1 protein, using recombinant molecular biological techniques provides a better approach to the development of sensitive and specific serological assays. Over the past 20 years, the baculovirus expression system has become one of the most popular vehicles for the production of recombinant proteins. The baculovirus technology underwent several advances during the recent years, including the development of a wide variety of transfer vectors and cloning methods, simplified recombinant virus isolation, easier quantification methods, the efficient application of cell culture technology, and the commercial availability of reagents (Kost et al., 2005). However, the manipulation involved in creating, titrating, and amplifying viral stocks for the conventional baculovirus expression of proteins remains a time-consuming and laborious methodology.

In this study, the expression of the L1 protein of HPV16 in insect cells using two plasmid and baculovirus systems was evaluated and compared in order to provide suitable antigens for the diagnostic tests and vaccines and accelerate the expression of L1 recombinant protein of HPVs. The reliability of purified L1 from both systems of plasmid and baculovirus to serve as antigens in serological tests were assessed and compared.

## MATERIAL AND METHODS

**Human Serum Samples.** Serum samples (n=42) were obtained from hospitalized patients during June 2010 and were stored at -70˚C until use. Full informed consent was taken from the patients at the time of sampling. The specimens and associated clinical data were collected and the specimens were anonymized by renumbering and removing all patient identifiers from the data before use in this study.

**Generation of DNA Fragment.**The entire gene sequences of HPV16 L1 from multiple isolates previously published in GenBank (http://www.ncbi.

nlm.nih.gov/sites/entrez?db=Nucleotide&itool=toolbar) were aligned using BioEdit Sequence Alignment Editor (Ibis Biosciences, Carlsbad, California, USA) and the primer sequences were designed from highly conserved regions. An NCBI Basic Local Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST/) search was performed to indicate any homology between primer sequences and all published sequences in GenBank. To generate specific vector-compatible overhangs, 5' CAG GGACCCGGT 3' and 5' GGC ACCAGAGCGTT 3' sequences were incorporated in the sense and anti-sense primers, respectively. Using HPV DNA extracted from a clinical sample as target, the HPV DNA region encoding L1 was amplified by PCR. The PCR products were purified using Microcon centrifuge method (Clear Diagnostic, Alamed, USA) and were analyzed by 2% agarose gel electrophoresis.

**Recombinant Plasmid Construction.** Ligation-Independent cloning (LIC) directional strategy was used for cloning. Briefly, the insert with overhangs prepared by PCR was treated with T4 DNA polymerase (Novagen, USA) in the presence of dATP (Novagen, USA). To create recombinants, the treated insert was annealed with the PIEx/Bac-3 3C/LIC vector (Novagen, USA) containing the hr5 (homologous region 5) enhancer and the ie1 (immediate early 1) promoter from Autographacalifornica nuclear polyhedrosis virus (AcNPV) (Luckow and Summers, 1988). Furthermore, the vector encodes an N-terminal His-Tag fusion sequence. The annealed complex was used to transform *Escherichia coli* (NovaBlueGiga Singles™ Competent, K-12 strain; Novagen, USA). In order to confirm the insertion of plasmid DNA, the colonies were screened by PCR following DNA extraction. Vector-specific primers (IE1 Promoter and IE1 Terminator, Novagen, USA) were used, alternatively, one vector-specific primer was used in combination with an HPV L1 gene-specific primer. The recombinant plasmid was purified using Mobius™ 200 kit (Novagen, USA) and was analyzed by 1% agarose gel electrophoresis.

**Plasmid Expression System.**The Sf9 cells (Novagen, Merck, USA), derived from *Spodopterafrugiperda*, were grown with serum-free medium (BacVector; Novagen, USA). These cells ($1x10^6$/ml) were transfected with 20 µg of recombinant plasmid DNA and 100 µl of Insect GeneJuice transfection reagent (Novagen, USA) according to the InsectDirect expression protocol (Novagen, USA). The cells were incubated at 28 °C with shaking at 150 rpm for 48 h.

**Baculovirus Expression System.** To generate recombinant Baculovirus, 1 ml BacVector medium, 5µl Insect GeneJuice, 100 ng BacMagic DNA (Novagen, USA), and 500 ng recombinant plasmids were assembled in a sterile tube. Instead of the recombinant plasmid for the negative and positive controls, TlowE buffer (10 mM TrisHCl, 0.1 mM EDTA, pH=8) and transfection control plasmid (Novagen, USA) were used, respectively. Following 30 min of incubation at room temperature, the mixture was added to the insect cells ($1x10^6$ cells/dish) and incubated at 28 °C. After 5 days, the recombinant baculovirus was harvested, amplified, and stored at 4 °C. To accurately determine the titer of the recombinant baculovirus, a plaque assay was performed. For protein expression, Sf9 cells were grown in a serum-free medium (BacVector Medium, Novagen, USA). The suspension cultures of Sf9 cells ($1\times10^8$ cells seeded in 50 ml medium) were infected at an MOI of 10. The cultures were incubated at 28°C with shaking at 150 rpm for 72 h.

**Protein Purification.** For 1 ml of the culture volume, 50 µl of Insect PopCulture Reagent (Novagen, USA) was added to the cells, followed by 0.5 µl Benzonase Nuclease (Novagen, USA). The mixture was incubated for 15 min at room temperature. The culture extract was centrifuged for 10 min at 15,000 g and the supernatant was saved. Afterwards, for $1x10^8$ infected cell pellets, 20 ml CytoBuster reagent (Novagen, USA) was added. The mixture was incubated for 5 min at room temperature, centrifuged for 10 min at 5000 g, and the supernatant was saved at 4 °C. Five hundred microlitres of Ni-NTA His•Bind Resin (Novagen,

USA) was added to the extracts. After mixing on a rocking platform for 1 h at room temperature, the mixture was loaded on the column and the retained resin was washed with 5 ml 1X Ni-NTA wash buffer. The target protein was eluted with 1 ml 1X Ni-NTA elute buffer.

**Protein Analysis**

**Immunofluorescence.** Twenty-five microliters of the transfected insect cells were applied to each spot of a PTFE-coated 15-spot slide (C.A. Hendley, Essex, UK). The slide was dried and immersed in cold acetone (-20 °C) for 5 min. After washing in sterile water, a 1:100 dilution of anti-HPV16 L1 monoclonal antibody was applied to each spot and was incubated at 37 °C for 30 min. Following washing, the slide was incubated with a 1:100 dilution of anti-mouse FITC-conjugated (Dako Ltd, USA) at 37 °C for 30 min and was examined by fluorescence microscopy at 400× magnification.

**SDS-PAGE, Western Blot, and Dot Immunoblot Analysis.** Samples were applied to a NuPAGENovexbis-Tris mini-gradient PAGE gel 4-12% (Invitrogen, USA) and run in SDS-Tris-glycerine buffer. Proteins were visualized by Coomassie Blue staining (Simply Blue, Invitrogen, USA). Afterwards, the proteins were electrophoretically transferred to a membrane (PVDF Filter paper sandwich, Invitrogen, USA) using the XCell blot module (Invitrogen, USA) at a constant voltage of 200v. Next, the PVDF membrane was immersed in a solution of 5% dry skim milk-PBS-Tween 0.1% for 1 h. After blocking, the membrane was incubated for 1 h at room temperature on a rotator in a 1:200 dilution of HPV positive human sera. The membrane was washed three times in PBS-0.1% Tween 20. Following washing, the membrane was probed with a 1:1500 dilution of HRP goat anti-human IgG (Abcam, USA). One tablet of 3, 3′-diaminobenzidine peroxidase substrate (DAB, Sigma) was dissolved in 15 ml of the detection buffer (0.1 M Tris-HCl pH=7.6, 0.1 M NaCl, 50 mM MgCl2) and 20 µl of hydrogen peroxide (30%) was then added. The membrane was placed in the substrate solution until the bands appeared (approximately 15 minutes). The membrane was rinsed in water and left to dry on paper towels. For the dot-blot assay, 30 µl of the sample was spotted on a 0.45 µm pore nitrocellulose membrane (Schleicher and Schuell, Germany). The samples were dried under vacuum using a dot-blot apparatus (Vacuum Filtration System, Minifold, SRC, 96 D, Schleicher and Schuell, Germany). Following air drying, the membranes were immersed in 5% skim milk and incubated with the primary and secondary antibodies as described for western blotting.

**Protein Yield Determination.** Protein yield was determined using a spectrophotometer with the UV lamp warmed up for 15 min at the wavelength of 280 nm. The samples (eluted protein) or blank (elution buffer) were pipetted into the center of quartz cuvettes. The cuvettes were filled with a volume of sample sufficient to cover the aperture through which the light beam passed (1 cm). The absorbance was calibrated at zero applying the blank sample and the absorbance of protein was measured. The wavelength was then adjusted at 260 nm and the absorbance was assessed as mentioned above. Protein concentration (mg) was calculated utilizing the following formula: $(1.55\times A_{280})-(0.76\times A_{260})$ (Stoscheck, 1990).

## RESULTS

**Construction and Generation of Recombinants.** The entire coding sequence of the HPV16 structural gene (L1) was cloned into the PIEx/Bac-3 3C/LIC vector. The resultant plasmids were used to generate recombinant baculoviruses, which were produced by the co-transfection of Sf9 insect cells with the vector and BacMagic viral DNA. The recombinant viruses were confirmed by PCR amplification.

**Expression of L1 in Sf9 Cells Culture.** The corresponding protein of HPV16 L1 was expressed in Sf9 insect cells using both the plasmid-based system (InsectDirect) and the baculovirus system (BacMagic). In order to examine Sf9 cells infected with recombinant baculovirus containing the L1 gene (BacMagic system) or transfected with the recombinant plasmid (InsectDirect system), an indirect immunofluorescence

assay using a monoclonal antibody directed against L1 protein was used. The assay showed that the fluorescence intensity in the cytoplasm of the cells infected with recombinant baculovirus was stronger than the cells infected with a recombinant plasmid (Figure 1, Panels A-B). The fluorescence intensity of the baculovirus-infected Sf9 cells increased as the infection progressed from 24 to 72 h (Figure 1, Panels C-E). At 72 h after infection, immunofluorescence was best observed in the cells with HPV-specific antibodies indicating the number of cells infected with the recombinants. Based on these observations, 72 h of incubation was used in the subsequent expression experiments. The immunofluorescence assay of non-infected insect cells revealed no fluorescence signal demonstrating the specificity of the antibody reaction with cells infected with recombinant baculovirus. Moreover, the whole-cell lysates were analyzed by SDS-PAGE. The antigenicity and identity of the recombinant L1 protein were confirmed by western blot and dot immunoblotting. A protein band with an approximate molecular mass of 58 KDa was observed in the cells infected with recombinant baculovirus (Figure 2). The specificity of the reaction was shown by the lack of reactivity of these sera with the negative control (non-infected Sf9 cells). The plasmid- and baculovirus-based expression systems took 5 and 15 days from cloning to protein production, respectively.

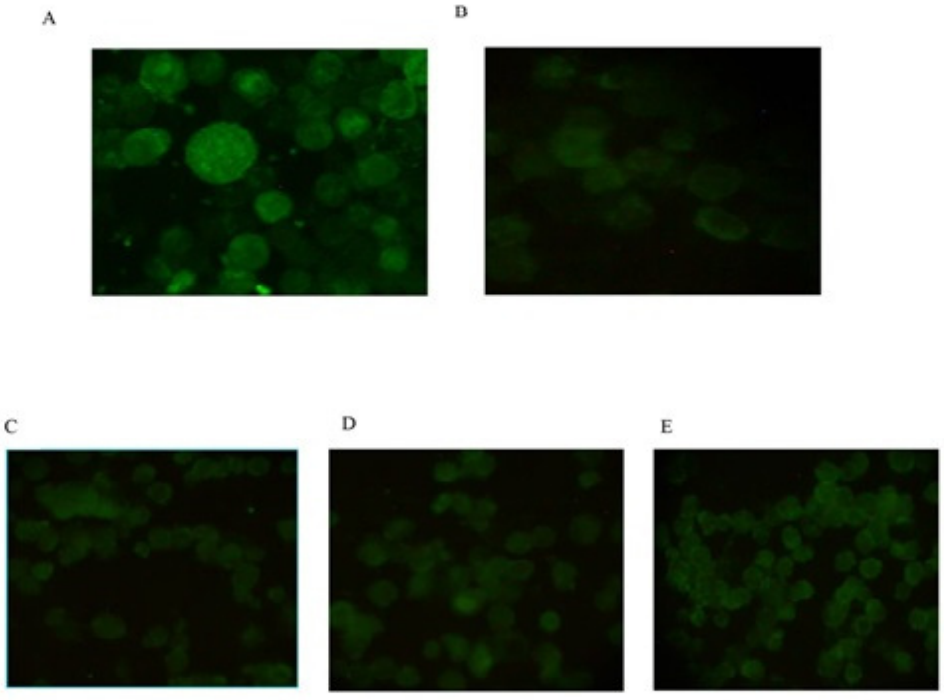


**Figure 1.** Immunofluorescence assay: a comparison between baculovirus L1 infected cells (BacMagic system) and plasmid L1 infected cells (InsectDirect system) (panels A-B) and a time course study of protein expression (panels C-E); Coverslips were stained using Papillomavirus Type 16 L1 monoclonal antibody followed by goat anti-mouse-FITC secondary antibody; Panel A: cells infected with recombinant baculovirus (BacMagic system), 72 h p.i.; panel B: cells transfected with recombinant plasmid (InsectDirect system), 72 h p.i.; Panels C-E: cells infected with baculovirus-L1 (panel C: 24 h p.i.; panel D: 48 h p.i.; panel E: 72 h p.i); the fluorescence intensity of the baculovirus-infected Sf9 cells increased as the infection progressed from 24 to 72 h.

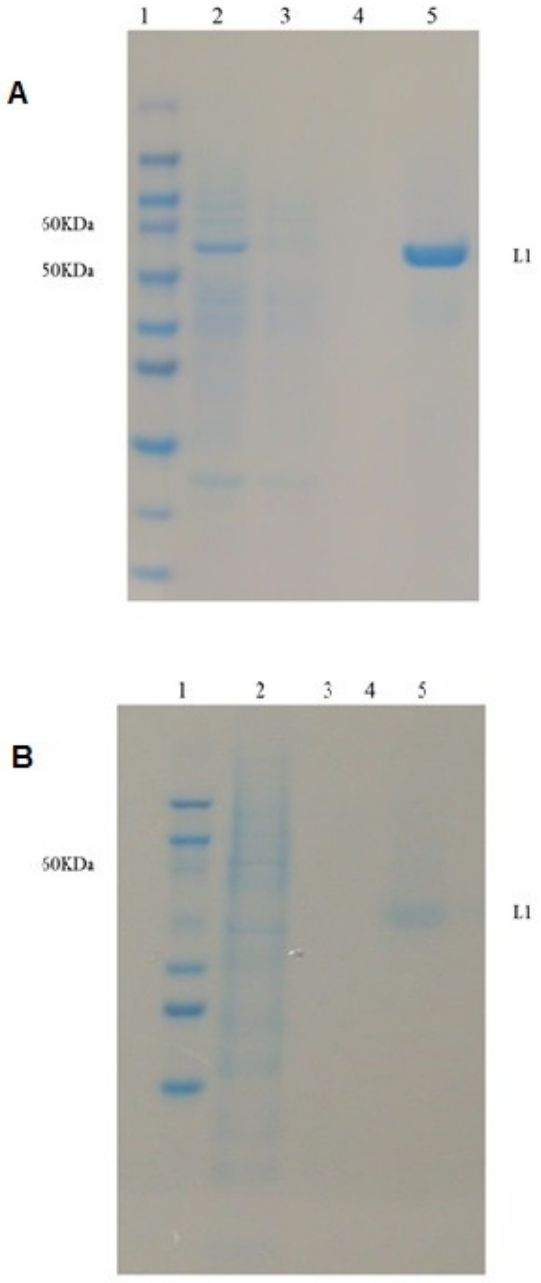


**Figure 2.** SDS-PAGE analysis of Papillomavirus Type 16 L1 protein expression and purification: a comparison of BacMagic baculovirus and InsectDirect plasmid system; the SDS-PAGE analysis of Ni-NTA-purified Papillomavirus Type 16 L1 protein in InsectDirect plasmid system (panel B) and BacMagic baculovirus system (Panel A). 1: marker; 2: cell supernatant; 3: flow-through; 4: wash; 5: Elute

**Purification of L1 Protein**. The expressed recombinant proteins were purified from Sf9 lysate using Ni-NTA resin columns. The SDS-PAGE analysis of purified proteins revealed that the recombinant proteins of the expected size (58 KDa) were produced in the InsectDirect and baculovirus systems. The results of SDS-PAGE demonstrated that a high degree (95%) of L1 protein purification was achieved using the baculovirus system (Figure 2, Panels A and B). The western blotting analysis of the lysates of Sf9 cells infected with the recombinant plasmids and baculovirus and purified proteins revealed a band with a weight of 58 KDa representing L1 protein (Figure 3, Panel A). Additional bands with lower molecular weights (27-37 KDa) were detected and considered as the degraded products of L1 protein. The antigenicity of the recombinant proteins was further analyzed by dot immunoblotting. The expressed protein was found to react with the virus-specific antibody (Figure 3, Panel B).

**Protein Yields Determination.** The yields of the purified proteins from the plasmid- and baculovirus-based systems (10 ml culture, 107 cells) had the ranges of 455-495 µg/ml and 1.44-1.6 mg/ml as analyzed spectrophotometrically, respectively.

## DISCUSSION

To allow HPV L1 recombinant protein expression in insect cells, a plasmid-based system (InsectDirect) and an improved baculovirus-based system (BacMagic system) were used. The aim of the present study was to determine the HPV L1 protein expression in insect cells using a plasmid expression system and an improved baculovirus expression system. The utilized LIC strategy provides a directional cloning strategy for the rapid creation of recombinants. The virus gene and promoter are carried by PIEX/Bac vector. The vector is dual-purpose and features the homologous region 5 (hr5) enhancer, immediate-early 1 (ie1) promoter for early phase expression, and AcNPV p10 very late promoter for late/very late phase expression from

Autographacalifornica nuclear polyhedrosis virus (AcNPV) for both of the plasmid- and baculovirus-mediated expression. Therefore, it is easy to switch between the different expression systems for meeting the requirements of protein production.

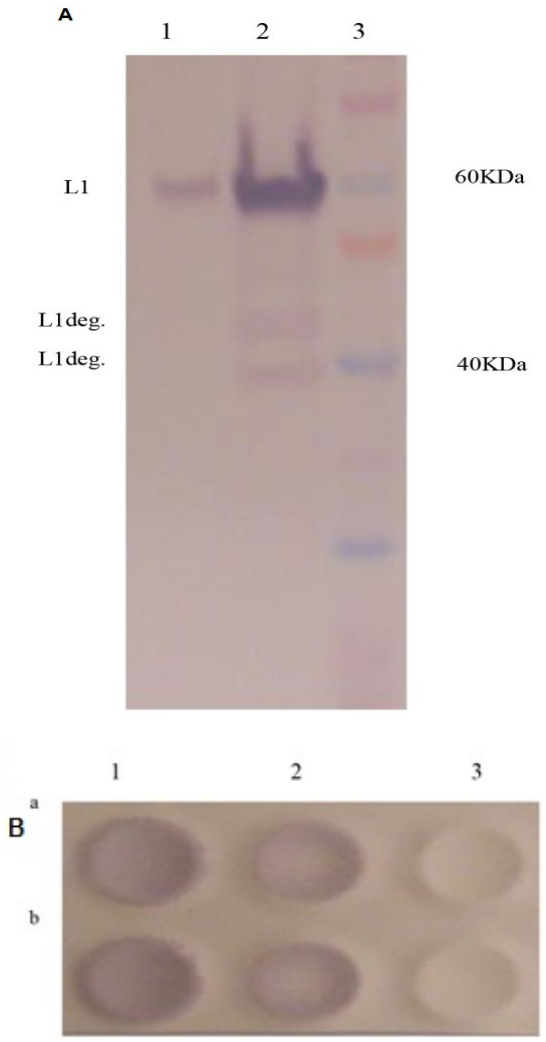


**Figure 3.** (A) Western blot analysis of recombinant Papillomavirus Type 16 L1 protein expression; the analysis of infected cell lysate and culture medium using anti-Papillomavirus Type 16 human sera; 1: non-purified cell lysate; 2: purified protein (72h p.i.); 3. marker.(B) Dot-blot analysis of L1 protein expression and purification in the BacMagic system; the analysis of Papillomavirus Type 16 L1 expression and purification by dot-blot using Papillomavirus Type 16 L1 rabbit polyclonal antibody followed by anti-rabbit IgG alkaline phosphatase antibody (lane a: 1 and 2. Infected cell lysate at 72hp.i, lane b: 1 and 2. Infected cell lysate at 96 hp.i, 3. non-infected cell).

The recombinant L1 protein expressed in both systems (BacMagic and InsectDirect) was characterized using immunofluorescence, SDS-PAGE, western blot, and dot-blotting. The immunofluorescence analysis of infected cells showed that the LI protein of HPV was successfully expressed in insect cells using both plasmid- and baculovirus- based systems. The assay demonstrated that the fluorescent intensity in the cytoplasm of cells infected with recombinant baculovirus was higher than cells infected with recombinant plasmid. The fluorescent intensity of the baculovirus-infected Sf9 cells elevated as the infection progressed from 24 to 72 h. According to these observations, an incubation period of 72 h p.i. was routinely used in subsequent expression experiments. An immunofluorescence assay using virus-specific antibodies of non-infected insect cells revealed no fluorescence signal indicating the specificity of the antibody reaction with recombinant infected cells. The HPV L1 with the molecular mass of about 58 KDa could be produced in insect cells from both plasmid (InsectDirect) and baculovirus expression systems (BacMagic) as observed in SDS-PAGE analysis. The western blot analysis of the lysates of Sf9 cells infected with recombinant plasmids and baculovirus showed a band with an apparent weight of 58 KDa representing L1 protein. Additional bands with lower molecular weights (27-37 KDa) were detected by western blot, which were considered to represent the degradation products of L1. However, no additional bands were visible after purification using His•Tag/His•Bind as analyzed by SDS-PAGE, likely indicating the absence of histidines on these degraded proteins. Western blot analysis revealed the presence of L1 in both cell lysate and culture. Virus-specific antibodies or human serum reacted well with recombinants in western blot and dot-blot analysis in both InsectDirect and BacMagic systems. The extraction and purification of the recombinant protein expressed in insect cells were simplified using detergent-based reagents (PopCulture and CytoBuster) that eliminated the need for

mechanical cell disruption. In His-Tag/His-Bind technology, L1 protein purification was based on the affinity between the ten neighboring histidines of the His-Tag sequence and an immobilized metal ion (Ni2+). The L1 protein that contained ten neighboring histidines was successfully bound to immobilize the metal ion (Ni2+) and eluted through the columns. This rapid, non-labor, and one-step process was amenable to the purification of proteins in their native state. His-Tag/His-Bind technology enabled the efficient purification of proteins from insect cells by completely eliminating ultracentrifugation steps normally required for cesium chloride or sucrose cushion purification methods and avoided the conditions of high ionic strength, which might negatively affect the stability of the L1. In most of the previous studies, HPV L1 protein was expressed using plasmid without His tags and had to be purified by cesium chloride ultracentrifugation, which was tedious and expensive (Chang et al., 1996; Chang et al., 1997; Goldmann et al., 1999). Here, the whole procedure of purification was only 3 h and the cost was much lower than the CsCl method. It could be concluded that the purification of L1 protein using Ni-NTA resin columns is convenient, time-saving, and less expensive. The SDS-PAGE analysis of the purified proteins showed strong bands with an apparent weight of 58 KDa representing L1. The Coomassie Blue stained SDS-PAGE gel revealed only a single band without any background indicating a high degree of purification. The presence of protein in the baculovirus system was indicative of obvious improvement in the amount of protein produced by this system, in comparison with that produced using the InsectDirect system. The molecular mass of plasmid-based expressed proteins was in good agreement with those of the baculovirus system-derived protein indicated by SDS-PAGE analysis. The result of western blot and dot-blot demonstrated that all the purified proteins were able to react with the virus-specific antibodies showing the antigenicity of purified L1 protein. The specificity of the reaction was confirmed by the lack of reactivity of these sera with the negative controls (i.e., non-infected cells). In the current study, the HPV16 L1 protein concentration was estimated by UV spectrophotometry at 280 nm. The yields of purified L1 protein from the plasmid and baculovirus systems (10 ml culture; 107 cells) had the ranges of 455-495 µg/ml and 1.44-1.6 mg/ml, respectively. The yield of protein achieved in the BacMagic system was considerably higher than the previous reports, which applied conventional baculovirus expression in insect cells (Li et al., 2003; Viscidi et al., 2003). The high yields of L1 protein were achieved by the expression of recombinant baculovirus containing fragments that encode the L1 gene under the control of baculovirus P10 very late promoter, compared to the expression of protein under the control of plasmid ie1 promoter in the InsectDirect system. As a result, for large-scale protein production, the baculovirus system might be preferred. On the other hand, the plasmid-based system is appropriate for the rapid screening of protein (from cloning to protein in 48 h). In the present study, the successful expression of the HPV16 L1 protein was achieved in both of the plasmid- and improved baculovirus-based systems in insect cells. Comparing to traditional baculovirus expression systems, the BacMagic system eliminates the time-consuming and laborious steps of recombinant baculovirus isolation. Moreover, the plasmid-based system improves the function of the baculovirus system by eliminating the time-consuming steps of recombinant baculovirus construction, screening, amplification, and plaque purification.

The BacMagic system is an appropriate system for large scale protein production and provides an alternative to the traditional baculovirus system. In addition, the InsectDirect system can provide a rapid expression system to check the successful production of a protein by a baculovirus system. Both the InsectDirect and BacMagic systems present remarkable savings in cost and time. The previous studies have developed immunoassays for HPV16 L1 protein expressed in baculovirus, *E. coli*, or yeast systems.

However, there is no report of an immunoassay for HPVS based on plasmid-derived L1.

## Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted article.

## Grant Support

The current research was supported by the Razi vaccine and serum research institute as a research grant with the code of 92120-18-18-2 for Bahman Abedi kiasari.

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