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In Silico Study, Design, and Expression of an Intranasal Dual Chimeric Vaccine for Indonesian-Based Norovirus GII-2 and Hepatitis B

Ernawati Arifin Giri-Rachman[1,2*], Marselina Irasonia Tan[1,2], Novia Syari Intan[1], Putri Ayu Fajar[1], Gladys Emmanuella Putri Wojciechowska[1,3], Rukman Hertadi[4], Debbie Soefie Retnoningrum[5†]

[1]*School of Life Science and Technology, Bandung Institute of Technology, Bandung 40132, Indonesia*
[2]*Nanoscience and Nanotechnology Research Centre, Bandung Institute of Technology, Bandung 40132, Indonesia*
[3]*Clinical Research Centre, Medical University of Bialystok, Bialystok, Poland*
[4]*Faculty of Mathematics and Natural Science, Bandung Institute of Technology, Bandung 40132, Indonesia*
[5]*School of Pharmacy, Bandung Institute of Technology, Bandung 40132, Indonesia*

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ABSTRACT

Hepatitis B virus (HBV) remains an important healthcare challenge, leading to liver diseases like cirrhosis and cancer. In response, we created a prophylactic and therapeutic HBV vaccine by integrating HBcAg and HBsAg from HBV genotype B into Norovirus (NoV) GII.2 P domain (PdomGII.2-HBV) for enhanced intranasal delivery. This vaccine also aimed to simultaneously prevent NoV infection, which causes gastroenteritis. Since the selected HBV epitopes have undergone extensive research and are tailored to the Indonesian population, this study focused on identifying NoV epitopes and assessing T cell epitopes coverage of the PdomGII.2-HBV for the Indonesian population. Following that, we expressed the PdomGII.2-HBV protein using *Escherichia coli* BL21(DE3) and employed a gentle solubilization technique for protein purification. Our *in-silico* analysis identified two B cell epitopes, along with 15 CD4+T cell epitopes and 35 CD8+T cell epitopes within the GII.2 P domain. These T cell epitopes cover 100% of the Javanese-Sundanese population's HLA allele variations, which constituted the largest demographic group in Indonesia. Subsequently, we successfully purified the presumed PdomGII.2-HBV protein, revealing a molecular weight of 39.5 kDa. Following the successful expression and purification of the presumed PdomGII.2-HBV protein, it is evident that this vaccine design has significant potential, warranting further study.

1. Introduction

Hepatitis B virus (HBV) is a significant concern in Indonesia, with a 7.1% medium-to-high prevalence rate and potential progression to severe conditions like liver cirrhosis and cancer (Muljono 2017). Existing antiviral medications may carry long-term risks of viral resistance and drug toxicity, making therapeutic vaccines an intriguing alternative (Fung *et al.* 2011; Akbar *et al.* 2013). A previous study demonstrated that a combination of commonly used antibody-inducing HBV surface antigen (HBsAg) and HBV core antigen (HBcAg), with its ability to activate cellular immunity, represents a potent therapeutic agent (Akbar *et al.* 2010). Combining the protective benefits of both antigens offers both therapeutic and prophylactic solutions.

To prevent sexual transmission of HBV, a prophylactic vaccine inducing mucosal immunity is crucial. Intranasal vaccination is a promising approach that triggers both mucosal and systemic responses within the Nasal-associated Lymphoid Tissue (NALT) (Birkhoff *et al.* 2009). It extends to other mucosal sites, including the reproductive tract (Holmgren and Czerkinsky 2005). It also provides extended protection and simplicity compared to intravenous methods (Birkhoff *et al.* 2009). To enhance the immunogenicity of HBcAg and HBsAg administered intranasally, Norovirus (NoV) P particle was used, offering a platform with multiple antigen presentation loops (Tan and Jiang 2012; Kocher *et al.* 2014). This unique property also generates protective antibodies against NoV (Tan and Jiang

† Deceased during preparation of the manuscript
* Corresponding Author
E-mail Address: erna_girirachman@itb.ac.id

2012), making it particularly relevant in Southeast Asia, where NoV outbreaks are a concern (Utsumi *et al.* 2017; Ruis *et al.* 2020). NoV infection prevention requires mucosal and systemic immunity (Green *et al.* 2020). Combining NoV and HBV vaccines in an intranasal dual vaccine can benefit chronic HBV patients, prevent sexual transmission, and provide NoV protection. Previous dual vaccines with P particles have shown promising protection against viral challenges (Tan *et al.* 2011).

The HBV epitopes were selected from HBV genotype B, and the NoV P domain used were derived from GII.2, as the dominant genotypes in Indonesia (Lusida *et al.* 2016; Utsumi *et al.* 2017). While the use of HBV epitopes has been studied extensively (Nursanty 2013; Romanò *et al.* 2015), the potential use of the GII.2 P domain as a vaccine platform for Indonesia HLA types remains largely unexplored.

Thus, this study aims to design the chimeric NoV GII.2 P domain-HBV (PdomGII.2-HBV) and assess the epitopes, particularly within the NoV GII.2 P domain. We investigate B and T cell epitopes within PdomGII.2-HBV, assessing its potential to trigger specific immune responses against NoV and establish immunological memory. Our analysis includes the P domain's T cell epitope coverage across Indonesian HLA allele variations, with a focus on the Javanese-Sundanese population, the largest demographic group in Indonesia. Furthermore, we have successfully expressed and purified PdomGII.2-HBV as an initial step toward characterizing its immunogenicity. This research contributes to the development of an intranasal dual vaccine for the benefit of the Indonesian population, targeting NoV and HBV.

2. Materials and Methods

2.1. Conservancy Analysis of NoV GII.2 P Domain

The selected NoV GII.2 P domain originates from NoV GII.P16-GII.2 (PDB ID: 5ysx), a structure with a known 3D configuration (Ao *et al.* 2018). It was then analyzed for conservancy based on the variability of 201 sequences of NoV VP1 protein with complete P domain region from the NCBI database, which was aligned using MEGA X (Kumar *et al.* 2018). Conservancy analysis was performed utilizing AVANA (Antigen Variability ANAlyzer) (Khan *et al.* 2017) with a threshold of 80%, considering the high diversity of NoV GII.2.

2.2. B Cell Epitope Prediction

Prediction of potential B cell epitopes involved a combination of two methods: ABCPred (Saha and Raghava 2006) and BepiPred 2.0 (Jespersen *et al.* 2017). Peptides of 16 amino acids were employed, with threshold values of 0.51 for ABCPred and 0.5 for BepiPred. Peptides suggested by both methods, either partially or completely, were selected, excluding those located in regions with less than 80% conservation. The selected B cell epitope candidates were further characterized using Parker hydrophilicity prediction (Parker *et al.* 1986) and Emini surface accessibility scores (Emini *et al.* 1985). Subsequently, the potential epitopes were mapped onto the 3D structure of the chimeric protein NoV GII.2 P domain-HBV using PyMOL Molecular Graphics System (Schrödinger and DeLano 2017).

2.3. T Cell Epitope Prediction

The HLA alleles used represented the Indonesian population, specifically the Javanese-Sundanese (Yuliwulandari *et al.* 2009). The NetMHCIIpan tool (Reynisson *et al.* 2020) was employed to predict peptide binding (15 amino acids) to HLA molecules from the DRB1 locus of HLA II alleles, with peptide selection based on a percentile ranking of <2. CD8+ T cell epitope prediction was conducted using two tools: NetMHCpan 4.1 (Reynisson *et al.* 2020) and NetCTLpan 1.1 (Stranzl *et al.* 2010) with a 9 amino acids peptide length. Subsequently, peptides meeting both criteria were subjected to further analysis.

2.4. Analysis of Predicted Epitope Similarity with Human Proteins

Similarity analysis was conducted by subjecting the peptides to BLASTp against the human proteome (Homo sapiens, taxid 9606) using predefined parameters (Gustiananda 2011).

2.5. Population Coverage Prediction

The selected peptides and their restricted HLA alleles were used for coverage prediction analysis using the Population Coverage prediction tool available through the IEDB server (http://tools.iedb.org/population/) (Bui *et al.* 2006). "Indonesia" was selected as the population and region of interest.

2.6. Chimeric NoV GII.2 P Domain-HBV Gene Construct

Following our previous research (Giri-Rachman *et al.*2023), the HBcAg epitope amino acid 18-27 (GenBank no. AKA94096.1) and "A" determinant HBsAg epitope amino acid 123-147 (GenBank no. AB466417.1) sequences were inserted to the P domain loop 1 (L71-D75) and loop 2 (Q156-D159) NoV GII.2 P domain. Additionally, we substituted arginine (R) with lysine (K) at position 186 to eliminate an unwanted thrombin-cleavage site. The design construct was synthesized as a gene fragment with a length of 1,083 bp in the pET-15b plasmid backbone. It is predicted to form a 39.5 kDa protein. The gene map was visualized using SnapGene® software (SnapGene 2024), while the 3D model was visualized using PyMOL Molecular Graphics System (Schrödinger and DeLano 2017).

2.7. Competent Cells Preparation and Plasmid Transformation

Chemically competent *E. coli* BL21 (DE3) cells were prepared using the CCMB80 method and were then transformed with pET15b-NoVGII.2-HBV plasmid, followed by inoculation onto solid Luria Bertani (LB) media supplemented with 100 µg/mL ampicillin (Green and Sambrook 2018). Transformant colonies were sampled and verified using the colony PCR method (Bergkessel and Guthrie 2013), employing KAPA2G FAST reagent (Sigma Aldrich) according to the manufacturer's protocol. The PCR results were visualized via agarose 1% agarose gel electrophoresis, and Sanger sequencing analysis was performed.

2.8. Expression of Chimeric NoV GII.2 P Domain-HBV Protein

5% overnight culture of *E. coli* BL21(DE3) containing pET15b-NoVGII.2-HBV was introduced into an LB medium supplemented with 100 µg/ml ampicillin. This culture was then incubated at 37°C with agitation at 200 rpm until the optical density (OD_{600}) reached a range of 0.5 to 0.7. Subsequently, 1 mM of isopropyl β-D-thiogalactopyranoside (IPTG) was introduced into the culture, and the incubation was continued under the same conditions for 28 hours. After that, the cells were harvested by centrifugation at 4°C for 5 minutes at a speed of 12,000 rpm.

2.9. Protein Expression Analysis

Bacterial cell pellets from both induced and uninduced cultures of *E. coli* BL21(DE3), and *E. coli* BL21(DE3) containing pET15b-NoVGII.2-HBV were analyzed using a 12% SDS-PAGE system (Mini-PROTEAN, Bio-Rad). Some of the cell pellets were resuspended in 500 µL of native binding buffer (NBB) (20 mM sodium phosphate, 300 mM NaCl, and 20 mM imidazole), added with 1 mg/ml of lysozyme, and sonicated to analyze both soluble and insoluble fractions. All samples were supplemented with a 1:1 sample buffer prior to SDS-PAGE analysis (Protein marker used: Pierce™ Unstained Protein MW Marker (Cat No.26610) (Thermo Scientific)/ Spectra™ Multicolor Broad Range Protein Ladder (Cat No.26634) (Fisher Scientific).

2.10. Protein Purification

PdomGII.2-HBV, primarily residing in the insoluble fraction, was solubilized by resuspending cell pellets with 3% Empigen BB detergent (EBB). After overnight incubation in a vertical shaker, it was then centrifuged at 4°C, 7,500 rpm for 20 minutes. The solubilized fraction (supernatant) was analyzed through SDS-PAGE purified via a nickel nitrilotriacetic acid (Ni-NTA) column (Thermo Scientific) integrated into the NGC chromatography system (Bio-Rad). The purification involved ten wash cycles with a specific wash buffer (20 mM sodium phosphate, 300 mM NaCl, 40 mM imidazole, 10% glycerol, and 1% EBB) and elution using elution buffer (20 mM sodium phosphate, 300 mM NaCl, 250 mM imidazole, 10% glycerol, and 1% EBB) followed by SDS-PAGE analysis of the solubilized protein product and fractions from the purification process.

3. Results

3.1. Analysis of Conservation, B Cell, andT Cell Epitope Prediction within NoV GII.2 P Domain

Conservancy analysis of NoV GII.2 P domain with AVANA showed that the amino acid 29-37, 108-130, 159-166, 173-180, and 221-229 have a conservancy of <80% (Supplementary Figure 1). Apart from these regions, the remaining areas are conducive to epitope prediction.

Only four of the eight B cell epitopes found within the NoV GII.2 P domain are conserved. These four

peptides underwent detailed characterization (Table 1). Epitopes 3 and 4 consistently exceeded predefined thresholds across all parameters.

NetMHCIIpan identified 15 CD4+ T cell peptides predicted to bind at least one HLA molecule from the HLA DRB1 allele in the Javanese and Sundanese populations (Supplementary Table 1). Additionally, the NoV CD8+ T cell epitopes prediction using NetCTLpan and NetMHCpan tools revealed 35 CD8+ T cell epitopes expected to bind at least one HLA molecule from the HLA A and/or B allele in these populations (Supplementary Table 2).

3.2. Analysis of NoV GII.2 P Domain Epitope-Human Protein Similarity and Population Coverage

Out of all the predicted NoV B and T cell epitopes, we identified five peptides that shared more than six amino acid similarities with human proteins (Supplementary Table 3). Additionally, based on the predicted NoV T cell epitopes and their corresponding restricted HLA alleles, we analyzed the population coverage predictions specifically for the Indonesian population, as described in Table 2. The analysis revealed that the HLA I and HLA II ligand coverage for the NoV GII.2 P domain within the PdomGII.2-HBV construct reached 99.93% and 99.15%, respectively. When considering the combined binding coverage of both HLA I and HLA II for the Javanese and Sundanese populations to the NoV GII.2 P domain within the PdomGII.2-HBV construct, a full coverage rate of 100% was achieved.

3.3. PdomGII.2-HBV Expression and Purification

Following the promising results of the described in-silico analysis (Section 3.1 and 3.2), we proceeded to the subsequent stages involving the expression and purification of the PdomGII.2-HBV protein. The gene construct illustration and the 3D model of the protein are shown in Figures 1A and 1B, respectively. The successful transformation was confirmed through PCR analysis, as depicted in Supplementary Figure 2, and the sequencing results (data not shown). The anticipated expression of PdomGII.2-HBV, with a protein size of 39.5 kDa, is visible as depicted in Figure 2. It is noteworthy that the protein primarily resided in the insoluble fraction. Afterward, the insoluble protein fraction was solubilized using Empigen BB detergent, leading to the successful purification, as shown in Figure 3.

4. Discussion

In this study, we constructed the PdomGII.2-HBV by integrating HBV epitopes, HBcAg and HBsAg, into the loops of the NoV P domain. The HBcAg amino acid

Table 2. Prediction of population coverage of NoV GII.2 P domain to HLA alleles variation in the Indonesian population

Parameters	Prediction on		
	HLA I	HLA II	Combined HLA I and II
Coverage	99.93%	99.15%	100%
Average Hit	9.2	3.43	12.63
pc90	5.26	2.25	8.37

Table 1. Characteristics of predicted NoV B cell epitopes. A value above the threshold indicates a positive result. Epitopes 3 and 4 consistently exceeded predefined thresholds across all parameters

Parameters	Epitope 1 TLPILTLGELSNSRFP	Epitope 2 GELQGTTQLQVSGICA	Epitope 3 PFDPSEDIPAPLGVPD	Epitope 4 VPTYTAQYTPKLGQIQ
Position in P domain GII.2	5-20	47-62	89-104	136-151
Position in PdomGII.2-HBV	5-20	47-62	94-109	141-156
Average bepiPred score (threshold: 0.5)	0.510	0.507	0.530	0.511
Standard deviation	0.027	0.057	0.028	0.022
ABCPred score (threshold: 0.51)	0.65	0.81	0.76	0.89
Average hydrophilicity score (threshold: 1.275)	-0.242	1.912	2.028	1.568
Standard deviation	2.477	1.524	1.763	1.025
Average surface accessibility score (threshold: 1.000)	0.786	0.754	1.114	1.506
Standard deviation	0.602	0.510	0.673	0.962

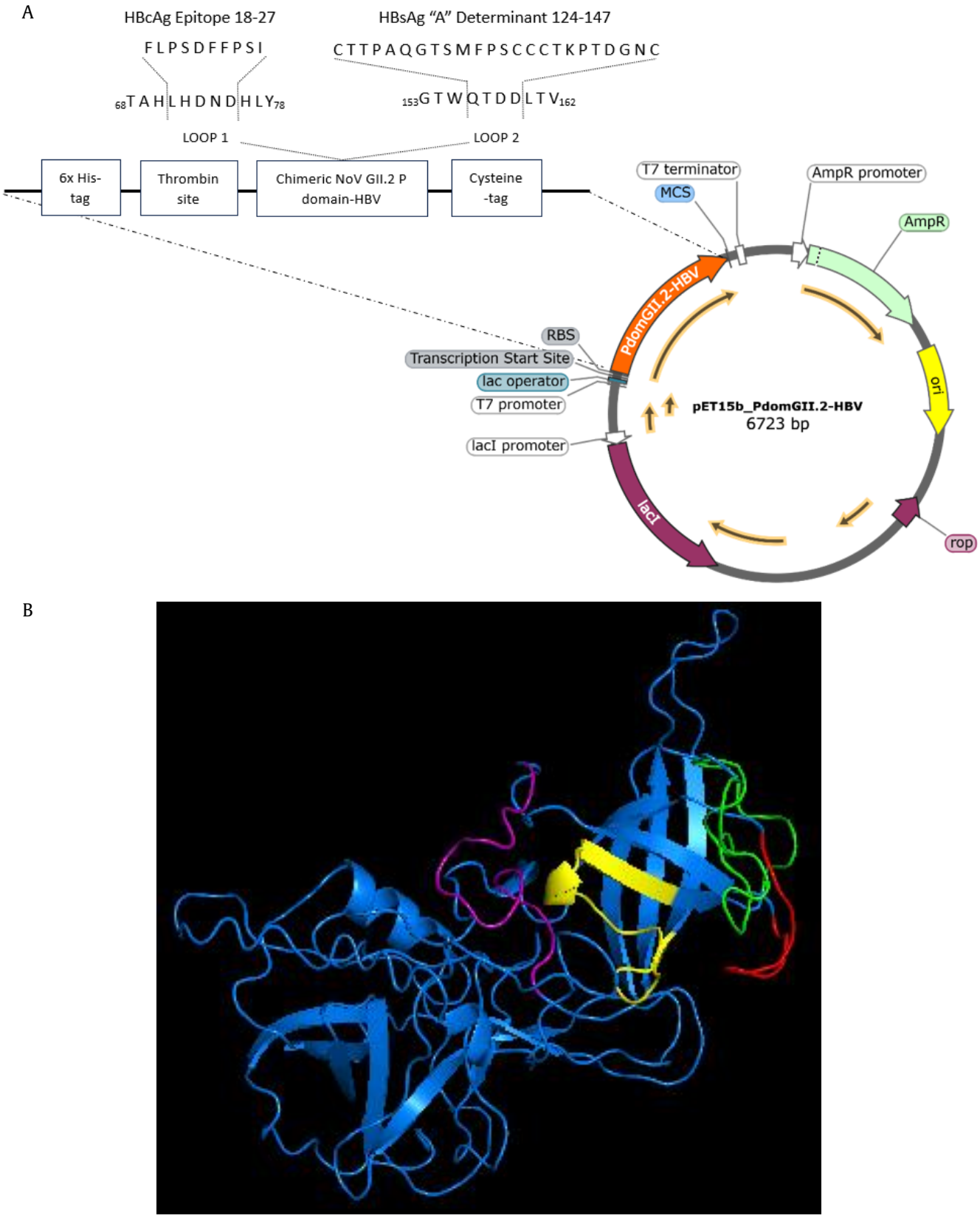

Figure 1. The gene construct and the 3D model of PdomGII.2-HBV. (A) The chimeric NoV GII.2 P domain-HBV (PdomGII.2-HBV) was constructed by inserting HBcAg and HBsAg into loop 1 (L71-D75) and loop 2 (Q156-D159), respectively. The pET-15b was used as a backbone for this gene construct. (B) The 3D structure of PdomGII.2-HBV. Green: HBsAg epitope, Red: HBcAg epitope, Purple: B cell epitope 3, Yellow: B cell epitope 4

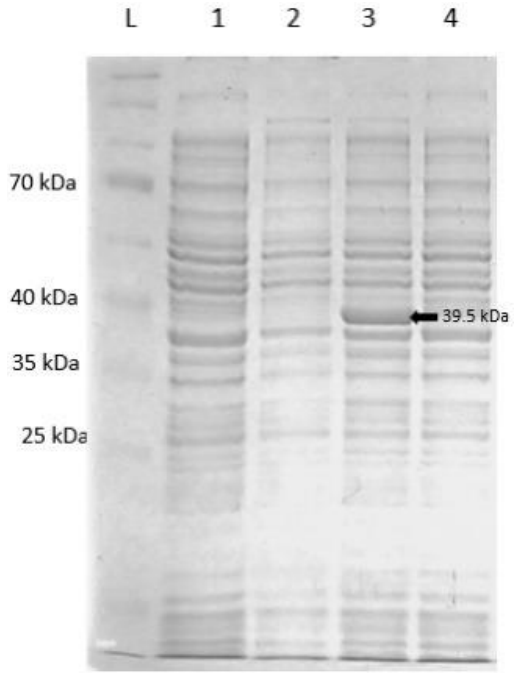

Figure 2. The crude protein profile of untransformed and transformed *E. coli* BL21(DE3) cells. The presence of a protein band at 39.5 kDa observed in lane 3 (IPTG-induced transformed cells) indicates successful expression of Pdom GII.2-HBV. 1: IPTG-induced untransformed cells, 2: uninduced untransformed cells, 3: IPTG-induced transformed cells, 4: uninduced transformed cells. L: spectra™ multicolor broad range protein ladder cat No. 26634 (fisher scientific)

18-27 (IEDB ID.16832) is a well-studied cytotoxic T cell epitope. Therapeutic polypeptides based on this immunodominant HBcAg 18-27 CTL can stimulate lymphocyte activation and proliferation, specifically induce CD8+ T cell expansion, and elicit robust HBV-specific CTL-mediated cytotoxicity in human PBMCs (Shi *et al.* 2004). This peptide is also highly recognizable by HLA alleles prevalent in the Indonesian population (Nursanty 2013). Additionally, the HBsAg "A" determinant (amino acid 124-147) is a conserved region across all HBV genotypes and serotypes, serving as the primary target for neutralizing antibodies elicited through natural infection or vaccination (Romanò *et al.* 2015). The inclusion of these two antigens is anticipated to offer both therapeutic and prophylactic benefits (Akbar *et al.* 2010; Romanò *et al.* 2015). To enhance the immune response triggered by the two antigens, the NoV P particle, formed by NoV P domain, was employed as a vaccine platform while also expected to protect against NoV infection (Tan and Jiang 2012). Our study on enhancing the efficacy of the HBV antigen using the NoV P domain is poised to be a breakthrough, particularly considering that vaccines for both HBV (therapeutic and prophylactic) and NoV are not yet readily available.

While both HBV and NoV can infect a wide range of populations, this vaccine candidate is primarily expected to benefit pregnant mothers who are at

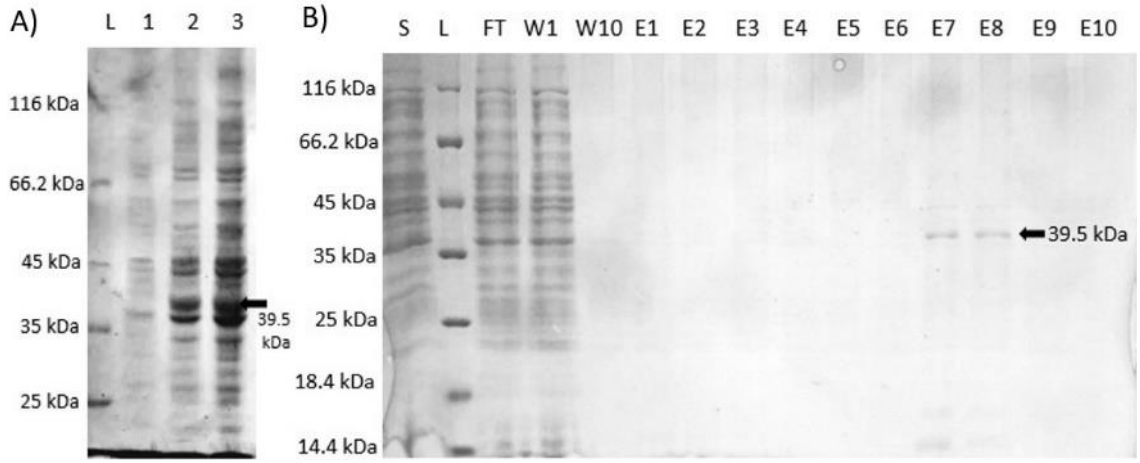

Figure 3. The protein profile of IPTG-induced transformed *E. coli* BL21(DE3) in crude form and purification fractions. The 39.5 kDa protein, which is expected to be Pdom GII.2-HBV, was observed mainly in lanes 2 and 3, showing its insoluble tendency. The protein was then obtained in E7 and E8. (A) The protein profile of IPTG-induced transformed *E. coli* BL21(DE3). L: unstained protein molecular weight marker 26610, 1: soluble fraction, 2: insoluble fraction (obtained after cell sonication), 3: crude cell. (B) The protein profile of the solubilized insoluble fraction and its purification results. L: pierce™ unstained protein molecular weight marker cat No. 26610 (thermo scientific). S: solubilized insoluble fraction, FT: flowthrough, W1: first wash fraction, W10: last wash fraction, E1-E10: elution fractions

risk of transmitting HBV to their children through undetected chronic HBV infection (Muljono 2017). Additionally, it is anticipated to benefit children and healthcare providers who are susceptible to both NoV and HBV infections (Nordgren and Svenson 2019). The intranasal delivery of this vaccine is also expected to stimulate mucosal immune defenses that extend to the reproductive area (Holmgren and Czerkinsky 2005), thereby preventing HBV sexual transmission and significantly benefiting sexually active individuals.

Building on our previous study (Giri-Rachman *et al.* 2023), the NoV type utilized in this research is NoV GII.2, a predominant type in Indonesia, making it particularly suited to protect the Indonesian population (Utsumi *et al.* 2017). This study aims to analyze its potential for use in the Indonesian population through in-silico analysis, followed by the expression and purification of the chimeric vaccine candidate.

We identified B cell and T cell epitopes within GII.2 P domain segments with >80% conservation. Utilizing ABCpred and BepiPred 2.0, we predicted B cell epitopes within Pdom GII.2, suggesting its potential to elicit humoral immune responses crucial for preventing infection. From these predictions, we selected 16 hydrophilic epitopes spatially positioned for recognition by B cell receptors (Emini *et al.* 1985; Parker *et al.* 1986). Among these, epitope 3 and 4, located in the P2 subdomain and exposed on the protein's surface during P particle formation (Tan and Jiang 2012), met all selection criteria (Table 1). Moreover, two of these epitopes overlap with those identified by Kobayashi *et al.* in 2016 within the NoV GII.2 strain Melksham/1944/UK (X81879).

We successfully identified 15 potential CD4+ T cell epitopes associated with NoV by focusing on peptide binding to HLA alleles. As shown in Supplementary Table 1, Peptides 4 and 5 were predicted to bind to HLA-DRB1*12:02, the most prevalent HLA II allele in the Javanese-Sundanese populations (37.8%). Additionally, peptide 13 had the most HLA allele bindings (7 alleles).

We used NetMHCpan and NetCTLpan to predict CD8+ T cell epitopes, considering peptide binding to specific HLA alleles and parameters, including proteasomal C-terminal cleavage and TAP transport efficiency. Following a sequence conservation-based selection, we found 35 CD8+ T cell epitopes (Supplementary Table 2). Peptides 2, 12, 19, 34, and 35 were predicted to bind to HLA-A*24:07, and peptides 5 and 6 were expected to interact with HLA-B*15:02, the most common HLA A (21.6%) and HLA B (11.6%) allele in Javanese-Sundanese populations, respectively. Peptide 33 had the most HLA allele bindings (12 alleles).

A similarity analysis between the identified epitopes and human proteins was conducted to evaluate the potential for autoimmune reactions, considering eight or more consecutive amino acids in the epitope-binding region that are similar to human proteins (Gustiananda 2011). Out of the epitopes, 4 showed 7-mer consecutive similarities, with only one epitope displaying up to 8-mer similarities with human proteins (Supplementary Table 3). Nevertheless, these similarities were not entirely located within the predicted 9-mer core binding area. Additional assessment may be required to ensure vaccine safety.

We compiled 15 CD4+ and 35 CD8+ T cell epitopes for assessing population coverage. The high coverage of the NoV GII.2 P domain within the PdomGII.2-HBV constructs binding with the analyzed HLA I and HLA II (>99%) implies that the identified T cell epitopes within the NoV GII.2 P domain effectively address the diversity in HLA alleles and offer protection for the Indonesian population, as represented by the Javanese and Sundanese groups. This aligns with the established criteria for good coverage, which is 90% (Fleri *et al.* 2017).

The expression of PdomGII.2-HBV within the pET15b-NoVGII.2-HBV vector was successfully achieved in *E. coli* BL21(DE3), producing a protein of approximately 39.5 kDa. However, the PdomGII.2-HBV protein exhibited insolubility and most likely formed inclusion bodies (Figure 3). This was due to the strategy of using high inducer concentrations and extended induction to boost protein expression. The challenge for *E. coli* in maintaining the integrity of rapidly synthesized proteins led to inclusion body formation (Singh *et al.* 2015). However, these inclusion bodies can be solubilized using the gentle detergent EBB (Lowthert *et al.* 1995).

After purifying the solubilized fraction, a protein band of approximately 39.5 kDa was identified in the seventh and eighth elution fractions, likely corresponding to our intended target, PdomGII.2-HBV. These findings, along with the solubilization outcomes, indicate the need to optimize the overall purification conditions. The presence of the target

protein in the seventh fraction suggests a potential issue within the purification column, possibly due to an excess of impurities or protein aggregation resulting from suboptimal detergent concentrations in the column (Bondos and Bicknell 2003). Future investigations should refine the protocol for expressing and purifying the chimeric protein NoV GII.2 P domain-HBV to increase protein yield. Additionally, the vaccine candidate should be tested for safety and efficacy before it is ready for public use.

In conclusion, this study has identified and confirmed the presence of two potential NoV B cell epitopes within the chimeric NoV GII.2 P particle-HBV construct. Furthermore, we have identified and validated 15 NoV CD4+ T cell epitopes and 35 NoV CD8+ T cell epitopes that exhibit conservation. The combined repertoire of these predicted T cell epitopes achieves complete population coverage for the HLA alleles found in the Indonesian population, as represented by the Javanese-Sundanese group. Additionally, we have successfully expressed and purified the chimeric NoV GII.2 P domain-HBV protein in *E. coli* BL21(DE3), yielding a protein with a molecular weight of 39.5 kDa. This promising result has demonstrated the potential of this vaccine design, warranting further development.

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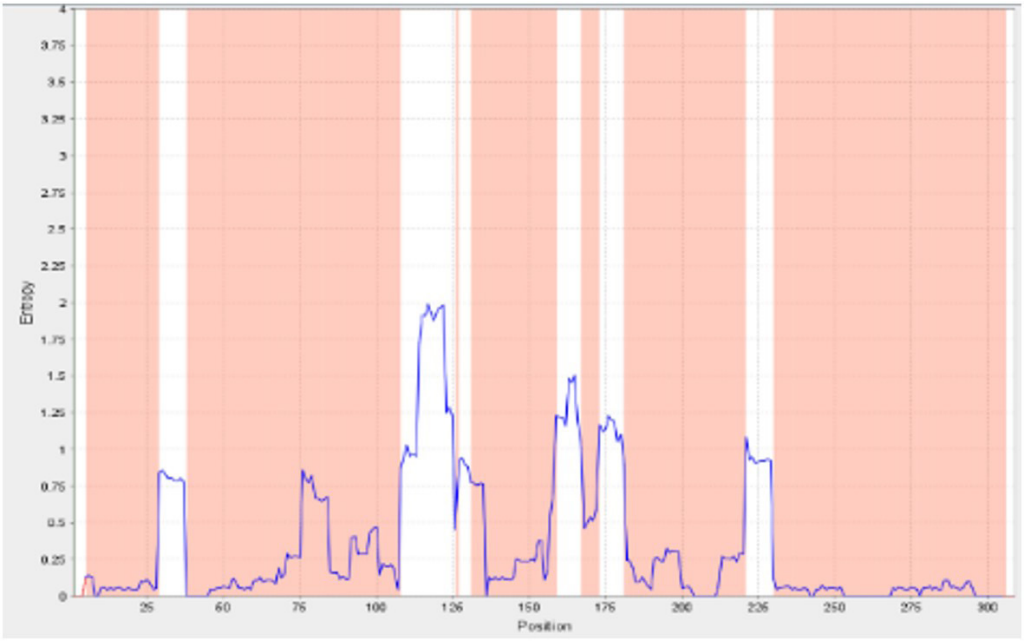
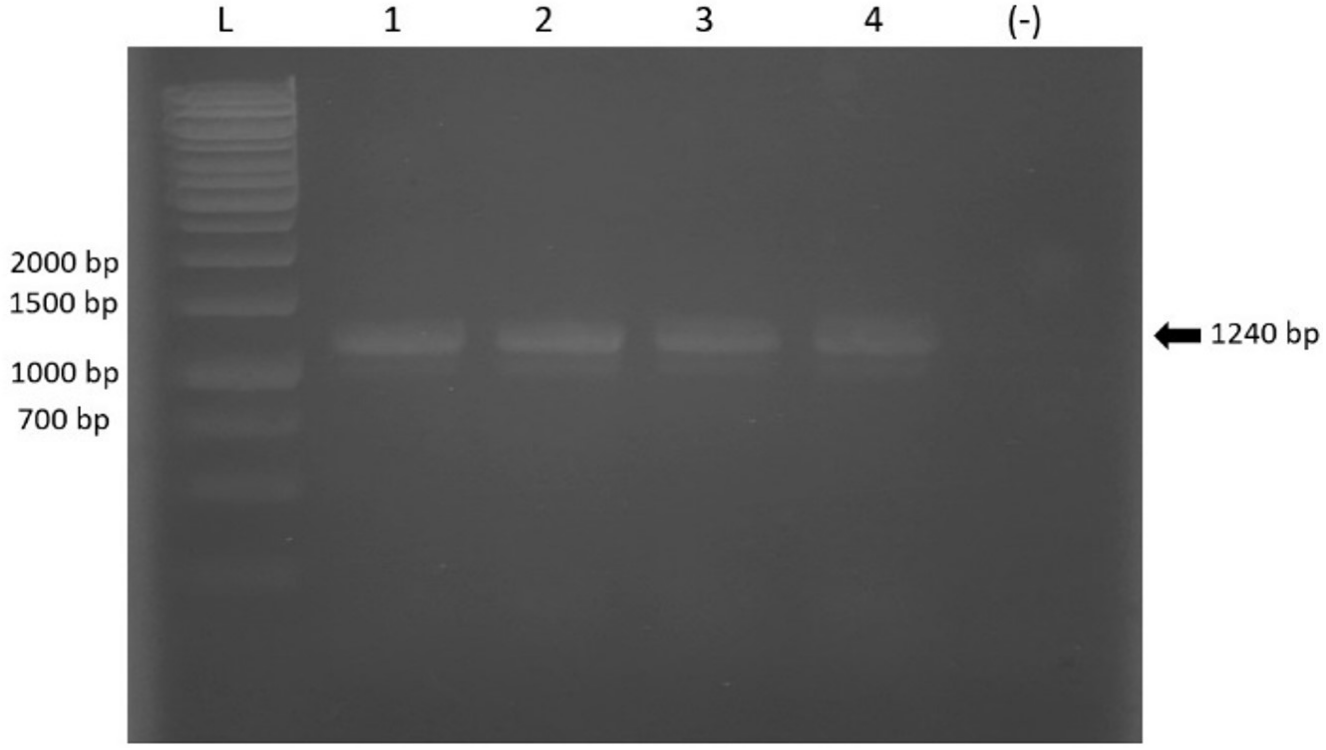
Supplementary Material



Supplememtary Figure 1. Conservancy analysis of NoV GII.2 P domain performed by AVANA. The area with <80% conservancy is marked by white color



Supplememtary Figure 2. The visualization of the PCR amplicon in colony PCR to confirm the success of *E. coli* BL21(DE3) transformation with PdomGII.2-HBV. Colony PCR results from 4 selected transformed *E. coli* BL21(DE3) colonies (1-4). Untransformed *E. coli* BL21(DE3) was used as a negative control (-). L: 1 kB DNA ruler (Thermo Scientific). A band of the expected size (1,240 bp) was observed in all four transformed colonies, whereas no band was detected in the negative control

Supplementary Table 1. Predicted NoV CD4+ T cell epitopes based on HLA binding prediction

Peptide*	Core binder	HLA allele
DGELQGTTQLQVSGI	LQGTTQLQV	DRB1*14:01, DRB1*14:04
QVSGICAFKGEVTAH	ICAFKGEVT	DRB1*15:01
HLYNVTITNLNGSPF	YNVTITNLN	DRB1*10:01
NVTITNLNGSPFDPS	ITNLNGSPF	DRB1*12:02
HDAVVPTYTAQYTPK	VVPTYTAQY	DRB1*14:05, DRB1*12:02, DRB1*13:02, DRB1*14:01, DRB1*14:04
VVPTYTAQYTPKLGQ	YTAQYTPKL	DRB1*01:01, DRB1*09:01, DRB1*10:01, DRB1*16:02
GALNLNTNLAPSVAP	LNTNLAPSV	DRB1*13:02
NTNLAPSVAPVFPGE	LAPSVAPVF	DRB1*07:01
PGERLLFFRSYIPLK	LLFFRSYIP	DRB1*15:01, DRB1*15:02
VQHFYQEAAPSMSEV	FYQEAAPSM	DRB1*01:01, DRB1*09:01, DRB1*10:01
EVALVRYINPDTGRA	VRYINPDTG	DRB1*04:06, DRB1*04:02, DRB1*15:01, DRB1*04:03, DRB1*15:02, DRB1*16:02
VRYINPDTGRALFEA	INPDTGRAL	DRB1*03:01, DRB1*13:02
RAGFMTVSSNTSAPV	FMTVSSNTS	DRB1*04:06, DRB1*07:01, DRB1*04:03, DRB1*09:01, DRB1*04:05, DRB1*10:01, DRB1*16:02
FMTVSSNTSAPVVVP	VSSNTSAPV	DRB1*13:02
ANGYFRFDSWVNQFY	YFRFDSWVN	DRB1*15:02, DRB1*16:02

*Peptides with partial similarity to human protein were written in bold

Supplementary Table 2. Predicted NoV CD8+ T cell epitopes based on HLA binding prediction and peptide processing in HLA I

Peptide*	HLA allele
LPILTLGEL	B*07:02, B*07:05, B*35:02, B*35:05, B*35:30, B*51:01, B*51:02, B*56:01, B*56:02, B*56:07
RFPVSIDQM	A*24:07, A*24:10
FPVSIDQMY	B*18:01, B*18:02, B*35:01, B*35:02, B*35:05, B*35:30, B*56:01, B*56:02, B*56:07
GELQGTTQL	B*13:01, B*37:01, B*40:01, B*40:02, B*40:06, B*41:01, B*44:03, B*48:01
LQVSGICAF	B*15:01, B*15:02, B*15:12, B*15:21, B*15:25, B*15:32
ITNLNGSPF	B*15:01, B*15:02, B*15:12, B*15:17, B*15:21, B*15:25, B*15:32
SPFDPSEDI	B*51:01, B*51:02, B*56:07
VPDFQGRVF	B*07:02, B*35:01, B*35:05, B*35:30
DAVVPTYTA	B*56:01
VVPTYTAQY	A*01:01, A*26:01, A*29:01
YTAQYTPKL	A*02:06, A*26:01, A*34:01
QYTPKLGQI	A*24:07, A*24:10
TPKLGQIQI	B*51:01, B*51:02
LGQIQIGTW	B*57:01
LTVNQPVKF	B*15:17, B*57:01, B*58:01
TEHFNQWVV	B*40:01, B*41:01
NLAPSVAPV	A*02:01, A*02:03, A*02:06, A*02:11
APVFPGERL	B*07:05
VFPGERLLF	A*24:02, A*24:07, A*24:10
FPGERLLFF	B*08:01, B*35:01, B*35:30, B*51:02
RLLFFRSYI	A*32:01
FFRSYIPLK	A*30:01, A*33:03
LPQEWVQHF	B*15:13, B*35:01, B*35:02, B*35:05, B*35:30, B*51:01, B*51:02
APSMSEVAL	B*07:02, B*07:05, B*35:02, B*56:02
SMSEVALVR	A*03:01, A*11:04, A*33:03, A*74:01
MSEVALVRY	B*15:17, A*01:01
SEVALVRYI	B*40:02, B*40:06, B*41:01, B*44:03
NPDTGRALF	B*35:01, B*35:05, B*35:30, B*56:02
GRALFEAKL	B27:06
ALFEAKLHR	A03:01, A33:03, A74:01
LHRAGFMTV	B*15:10
VVVPANGYF	B*15:17
VPANGYFRF	B*07:02, B*07:05, B*15:13, B*35:01, B*35:02, B*35:05, B*35:30, B*51:01, B*51:02, B*56:01, B*56:02, B*56:07
RFDSWVNQF	A*24:02, A*24:07, A*24:10, A*32:01
SWVNQFYSL	A*24:02, A*24:07, A*24:10

Supplementary Table 3. Similarities between predicted epitopes and human protein

Predicted epitope	Peptide	Human peptide	Human protein
NoV B cell epitope	PFDPSEDIPAPLGVPD	EDIPAPL	Putative protein product of Nbla02999; Methionine adenosyltransferase II (beta variant 2, beta isoform 1, beta isoform2, beta isoform CRA_d); Chain A and E Methionine adenosyltransferase II, beta
NoV CD4+ T cells epitope	NVTITNLNGSPFDPS	LNGSPDF	Immunoglobulin heavy chain junction region
NoV CD4+ T cells epitope	GALNLNTNLAPSVAP	NTNLAPS	Separin isoform X2 &X1, KIAA0165, isoform CRA_a, separase
NoV CD4+ T cells epitope	NTNLAPSVAPVFPGE	NTNLAPS	Separin isoform X2 &X1, KIAA0165, isoform CRA_a, separase
NoV CD4+ T cells epitope	ANGYFRFDSWVNQFY	GYFRFDSW/YFRFDSW	Immunoglobulin heavy chain junction region