I. INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a highly contagious and pathogenic coronavirus that caused a pandemic of acute respiratory diseases usually known as coronavirus disease 19' (COVID-19) (Hu et al., 2021). They are enveloped viruses having a non-segmented positive sense of single-stranded ribonucleic acid (ssRNA+) as their genetic material (Astuti, 2020). Approximately two-thirds of the SARS-CoV-2 genome occupies the replicase gene, referred to as open reading frame 1a and ab (ORF1ab) encoding for non-structural proteins, pp1a and pp1ab polyproteins. The remaining genome encodes for four structural proteins, namely, spike (S), envelope (E), membrane (M), nucleocapsid (N), and accessory proteins (Yadav et al., 2021). The S protein forms peplomers on the virion surface which facilitates viral entry. The N protein forms a complex with the virus's genomic RNA, forming a helical capsid (Diamond & Kanneganti, 2022; Yadav et al., 2021). Moreover, the N protein is involved in RNA synthesis, but the specific contribution to this process remains unclear (Wu et al., 2023).

Throughout the COVID-19 pandemic, multiple SARS-CoV-2 variants have emerged. Among them, the Alpha, Beta, Gamma, and Omicron variants, identified as variants of concern (VOCs), have demonstrated increased transmission rates relative to the previous one (Carabelli et al., 2023). According to Abavisani et al. (2022), these variants primarily result from mutations in the S protein. Given that the S protein plays a crucial role in viral entry by binding to the angiotensin-converting enzyme 2 (ACE-2) on the host cell, alterations in this protein lead to an increased binding affinity to the host receptor, consequently enhancing infectivity. In response to this, various types of vaccines have been developed and proven effective in eliminating the existing SARS-CoV-2 variants. However, the majority of these vaccines are designed to induce immune responses aimed at generating neutralizing antibodies against the S protein (Sadarangani et al., 2021). Therefore, these antibodies might lose their effectiveness in countering the emergence of novel SARS-CoV-2 variants, which

raises concerns about the long-term effectiveness of the vaccines and the potential for antibodies to evade detection.

When antibodies are no longer capable of preventing viral entry, another arm of adaptive immunity, specifically the T cell-mediated immune response, becomes essential in eliminating the virus (Mistry et al., 2022). Epitope-based vaccines specifically highlight the importance of T-cell mediated immunity where they utilize T-cell epitopes from conserved regions of different virus variants having antigenic properties (Martínez-Archundia et al., 2022). T-cells specifically recognize antigen/peptides presented by major histocompatibility complex (MHC) or human leukocyte antigen (HLA) in humans through the HLA Class I and II pathways (Janeway et al., 2001). HLA class I pathway processes cytosolic proteins into short peptides by the proteasome and transported into the endoplasmic reticulum (ER) via the transporter associated with antigen processing (TAP). In the ER, the peptide binds with the HLA class I molecule, forming a complex that is then presented on the cell surface for cytotoxic T cells (CD8+) recognition (Blum et al., 2013). In contrast, the HLA class II pathway processes exogenous antigens that are taken up through endocytosis. Following endocytosis, these exogenous antigens merge with lysosomes, where they undergo breakdown into short peptides through proteolysis. These peptides then bind to the MHC class II molecule, forming a complex. Subsequently, this complex is presented on the cell surface, specifically for recognition by helper T cells (CD4+) (Roche & Furuta, 2015).

As noted before, HLA molecules are crucial in presenting viral antigens to T cells. However, they are highly specific for certain peptide sequences presented by having a unique binding preference for antigenic peptides (Karnaukhov et al., 2022). Moreover, due to the extensive polymorphism within the HLA system, different populations possess different dominant HLA alleles (William, 2001). Indonesia, one of the countries with a diverse range of ethnicities, exhibits a unique genetic diversity across different geographical positions. This diversity correlates to diverse HLA alleles within the population (Pradana et al., 2020). According to Yuliwulandari et al. (2008), the top three most

predominant HLA class II alleles in the Indonesian population are HLA-DRB1*12:02, HLA-DRB1*15:02, and HLA-DRB1*07:01. However, the immune epitope database (IEDB) lacks data on SARS-CoV-2 peptide that can bind to these HLA alleles.

To create an effective peptide vaccine that stimulates a strong T-cell response, the initial step is to identify SARS-CoV-2 peptides that can bind to the prevalent HLA alleles in the Indonesian population. This is done through immunoinformatics study: epitope mapping. Previously, several HLA class II T cell epitopes derived from SARS-CoV-2 N protein have been predicted to be able to bind to the predominant HLA of the Indonesian population. Among those, three of them are $NP^{263-280}$, $NP^{352-371}$, and $NP^{387-406}$. Considering the high mutability of the S protein, the SARS-CoV-2 N protein is especially utilized due to its greater conservation and fewer mutations (Dutta et al., 2020). Additionally, N proteins from various coronaviruses exhibit high immunogenicity and are prominently expressed during infection (Cong et al., 2020).

The primary objective of this research is to validate the immunogenicity of the predicted HLA class II T cell epitopes ($NP^{263-280}$, $NP^{352-371}$, and $NP^{387-406}$) derived from the SARS-CoV-2 N protein. The concentration of antibodies against nucleoprotein: Anti-SARS-CoV-2 (N) IgG will be measured on the same study participants. Furthermore, the predicted peptides will be assessed for their potential to be B-cell epitopes. It hypothesized ($NP^{263-280}$, $NP^{352-371}$, and $NP^{387-406}$) are immunogenic towards the Indonesian population and the Anti SARS-CoV-2(N) IgG antibody are higher in individuals with COVID-19 history.