UNIVERSITI TEKNOLOGI MARA

DESIGN AND APPLICATION OF SARS-COV-2 DELTA VARIANT VIRUS-LIKE PARTICLES (VLPS) IN APTAMER-BASED DIAGNOSTIC ASSAY DEVELOPMENT

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ABSTRACT

The emergence and rapid evolution of SARS-CoV-2 variants present various implications towards public health and research. Viral propagation necessitates biosafety level 3 (BSL-3) containment, while the mutations harboured by the variants pose diagnostic challenges. These led to the exploration of virus-like particles (VLPs), which closely mimic the morphology of the virus but are non-infectious and safer to handle. While the diagnostics and therapeutics of COVID-19 predominantly rely on antibodies, aptamers are increasingly recognised as an alternative molecular recognition element. Aptamers are single-stranded nucleic acids that bind target molecules with high specificity and affinity. Their advantages over antibodies include lower production costs, faster synthesis, ease of chemical modification, and minimal batch-to-batch variability. Existing anti-SARS-CoV-2 aptamers can be further improved through rational design approaches, such as truncation, base substitution, 3'-polyadenylation, and multimerisation. In this study, aptamers were refined and employed in the development of an enzyme-linked aptasorbent assay (ELASA) for SARS-CoV-2 Delta variant VLPs detection, which were first generated as safe and functional surrogates for the native virus. Genomic analysis of Delta variant sequences isolated in Malaysia (August–December 2021) identified ten recurring spike protein mutations, which were incorporated into a recombinant DNA construct. This construct encoded the spike (S), membrane (M), and envelope (E) proteins, along with enhanced green fluorescent protein (EGFP), to enable the expression of VLPs. Following transfection into Vero 76 cells, VLP expression was confirmed by fluorescence microscopy, dot blot assay, and indirect immunoperoxidase staining. For the development of an ELASA, five aptamer candidates and their parent sequences were subjected to refinement and secondary structure prediction using the mFold web server. Direct ELASA screening revealed that the parent aptamer Apt25 and a truncated variant AptR-T exhibited the highest binding for the recombinant SARS-CoV-2 spike protein. Subsequent multimerisation of these high-affinity aptamers led to the development of a sandwich ELASA, utilising the homomeric variant Hom-RT as the capturing aptamer and Apt25 as the detection aptamer. This optimised sandwich ELASA demonstrated high specificity for the transfected cell lysate containing the SARS-CoV-2 Delta variant VLPs. The developed sandwich ELASA demonstrated a limit of detection (LOD) value of 14 nM. The successful construction of VLPs highlighted their utility as a non-infectious alternative to live virus for diagnostic assay development. The refinement of aptamers significantly enhanced their binding performance, indicating potential for improved diagnostic sensitivity and broader applications in therapeutic strategies as demonstrated through the novel ELASA.
(393 words)

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TABLE OF CONTENTS

CHAPTER 1
INTRODUCTION

1.1 Research Background

COVID-19 was first detected in Wuhan City, China, in December 2019, initially presenting as pneumonia symptoms with an unknown aetiology, before spreading globally and resulting in the pandemic, as declared by the World Health Organisation in March 2020 (WHO, 2020a). Since then, the virus, known as Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), has had a profound negative impact on the health system, global economy, and society, and the world is yet to fully recover from these devastating impacts (Clemente-Suárez et al., 2021; Calvo Ramos et al., 2024; Kim, 2025). As of July 2025, approximately 778 million positive cases had been reported globally, accounting for more than 7 million fatalities (WHO, 2025a). In Malaysia, over 5.3 million cases, contributing to over 37,000 deaths, were reported (MOH, 2025).

Due to its contagiousness and the potential for severe diseases, biosafety level 3 (BSL-3) facilities are required for the propagation of viruses (WHO, 2020b). Studies performed in any BSL-3 laboratory require adherence to biosafety protocols, which include the use of personal protective equipment (PPE), the potential application of a ventilation system and the utilisation of highly secure biosafety cabinets (CDC, 2021a). While PPE remains essential when handling potentially infectious specimens, it does not guarantee complete protection (Nguyen et al., 2020; Shaukat et al., 2020).

Since its emergence, SARS-CoV-2 has undergone continuous genetic evolution, giving rise to multiple variants. These mutations have led to increased transmissibility, immune escape, reduced vaccine efficacy, and diagnostic challenges, including false-negative results (Ridgway et al., 2023; Sarkar & Madabhavi, 2024). These undesirable situations have led to persistent and extensive studies, leading to advancements in diagnostics, vaccinology, and therapeutics (CDC, 2021b). Several robust technologies, including mRNA technology, virus-like particles (VLPs), antiviral development, and aptamers, have been widely improved and employed to combat the disease and establish preparedness for the potential emergence of pathogens.