Chapter 1

Introduction

1.1 Background

The human blood group consisted of four different types of phenotypes such as, A,B,AB and O, with the distinction between these subgroups lying on the expression of A and B antigens on the red blood cells (Wang et al., 2021). This was observed as A blood type expressed A antigens, B blood type expressed B antigens, AB blood type possessed both antigens and O blood types possessed none (Fathima & Killeen, 2025). The determination in expression of A and B antigen relied on the modification of precursor H antigen by glycosylation, this was shown as A antigen has N-acetylgalactosamine (GalNAc) attached towards the precursor H antigen and B antigen has galactose (Gal) attached towards the precursor H antigen (Dean et al., 2025). However as O blood type possesses none of these antigens, O type red blood cells displayed only precursor H antigen at the cell surface (Westhoff et al., 2018).

In order to determine the blood type of an individual, most healthcare institutions would generally conduct a serological test, which relied on the principle of hemagglutination of antigen with respective antibodies (Li & Guo., 2022). However this classic method may be inaccurate due to factors such as, different result interpretation and some reports of false results due to circulation donor red blood cells that may affect the results (Jacob, E.A., 2023). This is further proven as the serological blood typing method may not be suited for all patients, as patients reported to have blood disorders thus needed frequent transfusions such as, severe thalassemia or sickle-cell disease is likely to acquire false blood phenotyping with the use of serological testing (Sonker et al., 2023). Thus, genotyping and technologies such as Next Generation Sequencing (NGS) may aid in accurate ABO subgroup allele identification (Kim et al., 2021).

Although these technologies are highly sensitive and specific, this utilization of these methods is not suitable for general blood typing tests due to high cost and the application and machinery is not

widely available currently, making this method hard to access (Abbasi & Alexandrov, 2022). Thus, finding an alternative method that is highly sensitive and specific but still relatively affordable is critical in order to increase the accuracy of blood group phenotyping. With the advancement of technology, a highly sensitive and specific approach in diagnostics has been developed called Loop-Mediated Isothermal Amplification (LAMP) (Nam et al., 2023). This technology relied on the fundamentals of polymerase chain reaction (PCR), however, LAMP is more specific and sensitive due to up to eight target regions detected, and simple machinery due to isothermal temperature of around 60-65°C needed for LAMP (Yang et al., 2024).

1.2 Objective

The objective of this paper is to optimize multiple LAMP assays in ABO blood phenotyping with the use of reaction design alteration. Furthermore, this research aimed to use a non-invasive sample such as saliva due to ease of collection, thus increasing the convenience and comfort for blood phenotyping procedures . This paper also aimed to obtain a LAMP assay that was able to differentiate between blood groups by ensuring that there is a consistent huge gap in ct between for target blood group with non-targets. In addition the sensitivity of the LAMP assays in ABO group phenotyping was tested. Lastly, the stability of this LAMP assay was also tested in order to ensure the robustness of the LAMP reaction.

1.3 Hypothesis

The LAMP assays created to diagnose blood type A,B,O will be highly sensitive towards each blood type, this was observed as the LAMP assay would be able to detect as low as 10 copies/µl of sample and a good Cycle threshold (Ct) difference (>10) was observed between A,B,O samples for each assay. Lastly, the assay was expected to synthesize stable results even with the use of non-invasive saliva samples, thus the standard deviation of the results across different samples must be low.