CHAPTER I: INTRODUCTION

1.1. Background

Reports that the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the cause of the coronavirus disease 2019 (COVID-19) started back in December 2019 (Wu et al., 2020). Ever since then, COVID-19 became the epicenter of circumstances all over the world from the start of 2020 until this day. We are all currently living in a reality confined by those circumstances. Many people need to receive testing for SARS-CoV-2 to indicate whether or not they are carriers of the virus (symptomatic or asymptomatic). Most of us are required to stay at home and are forced to adapt to this new situation, for the safety of us as a collective. This has happened almost everywhere in the world. This is not the first time people are faced with a pandemic and likely not the last time as well.

The spread of viruses that are highly virulent such as SARS-CoV-2 can be contained if the detection occurs fast and efficiently from the beginning. It is true that we as a society perhaps have failed to recognize the severity of this virus from the beginning. Therefore, it is important to prevent such a thing from happening again. First and foremost, we need to search for the best solution in the frontline of these matters. One of the most important first actions is testing and tracing infected individuals.

The most common identification method currently used is quantitative reverse transcription polymerase reaction (qRT-PCR) of SARS-CoV-2 RNA. As we know, the polymerase chain reaction is a sensitive and specific method for amplifying DNA using the help of heat resistant Taq polymerase enzymes and specific primers. There is also the presence of probes in order to detect the results in real-time. Since SARS-CoV-2 is an RNA virus, it is required to convert its genome to DNA using reverse transcriptase (Bustin & Nolan, 2020). Reportedly, there are common cases of false positive and false negative results using qRT-PCR due to different primer-probe sets being inconsistent (Liu et al., 2020).

Figure 1. Illustration of RT-PCR thermal cycler

The widely used sample collection strategy is through the nasopharyngeal swab which is an uncomfortable procedure. Recently, saliva samples were chosen as an alternative for SARS CoV-2 detection because they can provide high detection rates for respiratory virus specimens (Vaz et al., 2020). It is also a non-invasive method that is more comfortable for patients, which reduces the risk of inducing sneezing or coughing and makes it safer for healthcare workers (Vaz et al., 2020). The use of saliva samples is one of the integral parts of this research due to its ease of collection. The fact that it is a non-invasive method, makes it a desirable option to look out for and consider as an alternative to the gold standard which is nasopharyngeal samples.

Reverse transcription loop-mediated isothermal amplification (RT-LAMP) is a potential alternative to the existing qRT-PCR and nasopharyngeal swabs methods. RT-LAMP is a nucleic acid amplification technique based on PCR technology which only requires one temperature. Thus, a thermal cycler is not required and a heating block can be used instead. RT-LAMP, as the name suggests, utilizes

the reverse transcriptase and a DNA polymerase that is unique due to having strand displacement activity along with being able to withstand varying temperatures. It is a self-priming technique which allows the chain reaction to continue (Dao Thi et al., 2020). This could provide a faster and cheaper test at the point-of-risk, the tests can be completed within 30-45 minutes at 65℃ (Lamb et al., 2020). This means that not many resources are needed or the tests can be performed in situations where there are constraints of resources. Pooling of the sample also helps in reducing the costs dramatically as multiple samples can be run in one reaction. In many underdeveloped countries or areas, RT-LAMP can be used without needing to worry very much about extra infrastructure and funding for thermocyclers unlike qRT-PCR. The most important resources are heating blocks and cold storage, which requires electricity.

Figure 2. Illustration of heating block used for RT-LAMP reactions

In this systematic review, the utilization of RT-LAMP as a screening test, not as a diagnostic test, was assessed. Screening tests are usually used on a population of asymptomatic individuals to assess how a disease might affect the population as a whole. It is a form of strategy to reduce the mortality or frequency of a certain disease within the population. Several examples of screening tests include pap

smear for cervical cancer and urinalysis for sexually transmitted diseases. Essentially, screening tests only provide two results between positive or negative and the ideal screening test would be where the result will show as positive only when the person tested actually has the disease (Maxim, Niebo & Utell, 2014).

Through this project we would like to summarize available studies on RT-LAMP detection of SARS-CoV-2 using saliva samples. Furthermore, we would like to compare their effectiveness and specificities.

1.2. Objectives

- To summarize available clinical studies on RT-LAMP detection of SARS-CoV-2 using saliva samples and compare their effectiveness and specificities.
- To recommend the most prospective RT-LAMP based SARS-CoV-2 detection method that is cheap, accurate, and high-throughput

1.3. Research Scopes

This research is going to be focused around analyzing various RT-LAMP methods that are available in databases such as PubMed, Sciencedirect, and Google Scholar. Studies that are analyzed are the ones published in 2020 until 2022. Comparisons will be made in order to achieve the objectives of the research.