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Reverse Transcription Loop-Mediated Isothermal Amplification in Detecting HIV-1 Infection: A Systematic Review

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ABSTRACT

Different diagnostic methods have been developed to detect the human immunodeficiency virus 1 (HIV-1) throughout the years. As science progresses, new methods which may be better than the traditional methods, such as loop-mediated isothermal amplification (LAMP), were invented. This systematic review aims to investigate RT-LAMP's performance in diagnosing HIV-1 in terms of its specificity and sensitivity. Relevant studies published between January 2000 to April 25, 2022, involving the sensitivity and specificity of the detection of HIV-1 in human blood samples using RT-LAMP were collected. Articles were identified through a search of PubMed and ScienceDirect databases. Out of 664 articles screened, eight articles were included in the study with consideration of established inclusion and exclusion criteria. A highly variable diagnostic value for RT-LAMP was observed in the systematic review as seen in its sensitivity values ranging from 53.85% to 100%. The specificity values, however, had a smaller range of 94.1% to 100%. Thus, the authors conclude that RT-LAMP might not yet be a valuable tool in the diagnosis of HIV-1.

KEYWORDS: HIV-1, RT-LAMP, Sensitivity, Specificity

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1 INTRODUCTION

The human immunodeficiency virus (HIV) is considered as a significant challenge in the field of public health in the span of a century. The current worldwide epidemic is caused by HIV-1 or human immunodeficiency virus type 1. HIV-1 belongs to the Lentivirus genus in the Retroviridae family. The acquired immunodeficiency syndrome or AIDS is caused by this virus. Its life cycle includes the following: (1) fusion and entry, (2) reverse transcription, (3) integration and translation, (4) viral protein formation, and (5) assembly. Notably, once it enters the host through mucosal surfaces and goes to the bloodstream, it attaches to the CD4+ T-lymphocyte. The virus is cleared from the blood through the lysis of infected cells brought about by the host's humoral and cellular responses, but this normally does not put an end to viremia. Once the infected CD4+ T-lymphocytes die, it will deplete CD4+ T-lymphocytes leading to a progressive dysregulation of the host's immune system in a span of years up to decades. This virus coexisting with opportunistic infections can ultimately hasten the host's death (Cassis-Ghavami et al., 2009; Catallozzi, 2008).

As per the recommendations of the World Health Organization (WHO), HIV virological assays that are designed for clinical diagnosis should have a minimum sensitivity of 95% but ideally more than 98% and a minimum specificity of 98% or more under ideal laboratory conditions.

Some of the most commonly used techniques to detect human immunodeficiency virus type 1 infections are enzyme-linked immunosorbent assay (ELISA), p-24 antigen test, and polymerase chain reaction (PCR). Each with its advantages and disadvantages.

Enzyme-linked immunosorbent assay (ELISA) is recognized to be the gold standard of immunoassays. ELISA is among the first-generation HIV antibody tests which used viral lysate antigens and only detected the IgG antibody of HIV-1. Currently, a fourth-generation ELISA is used, which can detect both HIV antibodies and p24 antigens, which makes early detection possible. It can identify specific protein antibodies and antigens even with small amounts of test samples. For HIV, the specimen of choice is blood or saliva. ELISA is used as a rapid antibody screening test and is accompanied by Western blot to be diagnostic. In addition, ELISA has 100% sensitivity as well as 99.9%-100% specificity when tested on 9,150 specimens (Alexander, 2016; Alhajj & Farhana, 2021; Yeom et al., 2006). ELISA's high sensitivity has its downfalls; it can cause false-positive results, which have been reported in different populations such as women who recently gave birth, people who were recently vaccinated against influenza or hepatitis B, patients who underwent multiple blood transfusion, patients with autoimmune diseases, patients with cirrhosis due to alcohol use, and patients with malaria and dengue infections (Wai & Tambyah, 2002). Hence, the protocol of confirming positive results with Western blot is recommended.

Western blot is a method that is used to separate and identify proteins. This method uses a mixture of proteins that will be separated based on their molecular weight, therefore allowing researchers to identify them by their type through gel electrophoresis (Mahmood & Yang, 2012). This method is used to confirm the presence of HIV-1 antibodies from licensed enzyme immunoassay (EIA) kits. Positive ELISA tests use Western blot as a confirmatory test and have a 98% sensitivity and specificity (Cordes et al., 1995). The Western blot or immunoblot assay may display up to nine bands in the electrophoresis to confirm the presence of HIV-1 proteins (Jackson et al., 1997). The ELISA-Western blot algorithm has been used for a long time. There are cases where the Western blot fails to detect an acute HIV infection, which delays the patient's antiretroviral therapy. For this reason, healthcare providers should be open to new and improved diagnostic methods for HIV (Medina-De la Garza et al., 2021).

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Antigen assays are also helpful in identifying the presence of HIV. The detection of HIV-1 p-24 antigen is an example of an antigen assay and it can detect HIV-1 after 16 -17 days of exposure to the virus. This test uses a monoclonal antibody in order to capture the HIV-1 p24 antigen which will then be detected by a another antibody. The result of the entire process will be in the form of a colorimetric readout. The p-24 antigen test has a specificity that can be up to 99.92%, while its sensitivity can be up to 99.0%. However, the p-24 antigen test will no longer become effective after 45 days from the onset of infection. Thus, antigen tests that detect HIV-1 p24 should be performed with a non-reactive serological screening test, and not as a stand-alone test (Weiss & Cowan, 2004).

Polymerase chain reaction (PCR) is currently the gold standard test in HIV screening. It is a nucleic acid test in which reverse transcription of HIV RNA occurs, and DNA copies are replicated through a continuous process of repetitive reactions. Synthetic primers that complement the border of each side of the area of interest are used for DNA PCR amplification. In theory, PCR can double the number of nucleotide areas of interest with each cycle. This doubling process will lead to a sufficient amount of DNA used for analysis (Weiss & Cowan, 2004). Theoretically, PCR tests are said to have 100% sensitivity and specificity. However, in clinical practice, HIV-1 DNA PCR assays appeared to have 96-99% sensitivity in the eclipse phase (3-7 days from exposure and infection), where viral DNA is within undetectable quantities (Lakshmi, 2011).

Nucleic acid amplification is a very valuable discovery in science. Nucleic acid amplification has been used in detecting infectious diseases and biotechnology, food quality control, and environmental studies. PCR has been widely used in detecting infectious diseases throughout the years because of its simplicity and reliability (Wong et al., 2018). It requires a high-precision thermal cycler since this process requires heat to denature the targeted double-stranded DNA to become single-stranded to initiate DNA synthesis. However, it is not accessible to private clinics and small laboratories because it requires sophisticated equipment. Throughout the years, scientists have been developing new amplification techniques that can overcome the limitation of PCR. Notomi et al. (2000) discovered an isothermal amplification technique that has the ability to amplify DNA efficiently, rapidly, and with high specificity.

The loop-mediated isothermal amplification (LAMP) is a new molecular technique known for its sensitivity and specificity toward the target DNA because it is capable of detecting six sequences of a target DNA using four primers and a DNA polymerase with strand displacement activity. LAMP consists of two different steps: non-cyclic and cyclic steps. The cyclic steps use an inner primer that holds the sense and antisense strand sequences of the target DNA to initiate LAMP (Sahoo et al., 2016). The strand of DNA primed by an outer primer will release a single-stranded DNA that will serve as the template for DNA synthesis. This template will be primed by the second inner and outer primers that will hybridize to the other end of the target, producing a stem-loop DNA structure. Another inner primer will be hybridized to the loop of the product and will initiate displacement DNA synthesis, creating the original stem-loop DNA and a new stem-loop DNA that is twice as long. This cycling reaction process will continue and accumulate 109 copies of the target in under an hour in an isothermal condition between 60°C and 65°C, an amount much higher than what PCR can produce. The end products are stem-looped DNA with inverted repeats of the target and multiple loops formed by annealing between inverted repeats in the same strand. In theory, LAMP is highly sensitive because it can recognize the target DNA by six distinct sequences, followed by four direct sequences expected to amplify the target (Notomi et al., 2000). This essential quality of LAMP is highly significant to the medical community as well as the general public, as it aids in augmenting the assurance of HIV-1 diagnosis. As HIV testing is normally equipped with highly purified virus antigens (Kimmig, 1990) and

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diagnostic algorithms, the emergence of LAMP would only exemplify virus recognition, especially in different instances like seroconversion.

Point-of-care (POC) rapid nucleic acid amplification testing (NAAT) methods that are easy to interpret, have a rapid turnaround time, do not require complicated equipment, and demonstrate high sensitivity and specificity are an exemplar to detect acute HIV-1 infection as early and as accurately as possible. An HIV-1 RT-LAMP that is capable of RNA and DNA detection in one reaction tube simultaneously has been evaluated for use in the laboratory as a rapid NAAT method. It exhibited promising results such as detecting the infection one to three weeks earlier in seroconverting patients compared to an antibody test. Aside from being a rapid POC NAAT, it can also be an option in certain laboratory settings because of its lower cost and faster turnaround time. This method has the potential to become a supplemental or confirmatory test alongside other methods (Rudolph et al., 2015).

The incongruence of the findings of primitive records and the absence of any systematic review on the diagnostic performance of RT-LAMP on HIV-1 calls for the need to summarize the existing findings of all relevant literature to provide more accessible evidence for professionals. The results of the accumulated studies will be summarized in the first systematic review on the performance of RT-LAMP in the detection of HIV-1 infection.

2 METHODS

2.1 Eligibility criteria

Table 1 Inclusion and Exclusion Criteria according to PICOS ("Population, Intervention, Comparison, Outcomes, and Study Design")

Parameter	Inclusion Criteria	Exclusion Criteria
Population	Blood samples of patients aged 0 to 99, both sexes	No HIV-1 positive blood samples
Intervention	Loop-mediated isothermal amplification method and its variants	No loop-mediated isothermal amplification and its variants utilized
Comparison	Not applicable	
Outcomes	Detection of HIV-1, sensitivity, specificity, true positive, true negative, false positive, or false negative	No HIV-1 detected, without sensitivity, specificity, true positive, true negative, false positive, and false negative
Study design	Case-control, cohort, case report, and experimental studies Published between 2000-2022	Animal studies, literature review, expert opinion, letters to the editor, conference reports, and cross-sectional studies Published before 2000 Papers not published in English

Table 1 shows the PICOS utilized to specify the inclusion and exclusion criteria of the primitive records in the systematic review. The study must include a population of blood samples of patients within the range 0 to 99 years old of both sexes, have used the loop-mediated isothermal amplification method as the diagnostic method, and have reported the sensitivity, specificity, true positive, true negative, false positive, or false negative in the clinical diagnosis of HIV-1 infection. The study must be a case report, case-control, cohort, or experimental study published from January 2000 to April 25, 2022. Studies that did not use HIV-1 positive blood samples and LAMP and its variants as the diagnostic method were excluded. In addition, studies without specificity, sensitivity, true positive, true negative, false positive, and false negative as variables were also excluded. The review also excluded animal studies, literature reviews, expert opinions, letters to the editor, conference reports, and cross-sectional studies. The researchers also barred research articles published before January 2000 and articles not published in English from being included. This systematic review followed the “preferred reporting items for systematic reviews and meta-analyses” (PRISMA) guidelines (Page et al., 2021).

2.2 Information sources

The researchers searched PubMed and ScienceDirect databases for research articles that will be included in this systematic review. The research articles were collected from both databases on October 16, 2021, and were screened on October 20, 2021. The articles were updated and screened once more on April 25, 2022.

2.3 Search strategy

For both databases, keywords, and filters were utilized in searching for articles. The keywords were typed as ("LAMP" OR "loop-mediated isothermal amplification") AND ("HIV-1") AND ("Sensitivity" OR "Specificity" OR "TRUE NEGATIVE" OR "TRUE POSITIVE" OR "FALSE NEGATIVE" OR "FALSE POSITIVE"). The articles were filtered from January 1, 2000, to April 25, 2022, and only those categorized as research articles and case reports were taken for screening.

2.4 Selection process

The researchers decided which studies to include and exclude in the systematic review by utilizing the constructed PICOS. Seven independent reviewers manually screened 94 to 95 records out of the 664 primitive records. The journals gathered were evaluated by the article title, body, and full text.

2.5 Data collection process

From the eight qualified reports, two reviewers collected data from each report independently. Data were deliberated among the proponents by their appropriateness and relevance to the study before synthesis.

2.6 Data items

The results collected from the studies included in this paper are the clinical sensitivity and specificity of LAMP in detecting HIV-1 infection. In considering the common factors of the gathered studies, the researchers decided to collect the said results because these results

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were relevant to answering the objectives of this systematic review paper. It was maximized to provide an excellent quality output.

The researchers extracted information such as the author's name, publication year, study design, number and type of sample, and the sensitivity, specificity, true positive, true negative, false positive, and false negative results of the diagnostic methods used. These parameters were used to evaluate the diagnostic performance of the reverse transcription loop-mediated isothermal amplification (RT-LAMP) in detecting HIV-1 infection.

3 RESULTS

A total of 664 primitive records were screened using the research parameters indicated in the PICOS of the study, 643 of which were retrieved from ScienceDirect, while the remaining 21 were retrieved from PubMed (Figure 1). Upon screening, seven articles were removed as duplicates. A further 649 articles were excluded from the list due to the following reasons: articles diagnosing a different condition (n=29), articles not using human blood (n=5), articles that used a method other than RT-LAMP (n=600), and articles that did not discuss the specificity, sensitivity, true positive, true negative, false positive, or false negative in the study's results (n=10), articles that were not written in English (n=3), an article from a book chapter (n=1), and an abstract only article (n=1). After screening, the researchers retrieved eight articles to be included in the review.

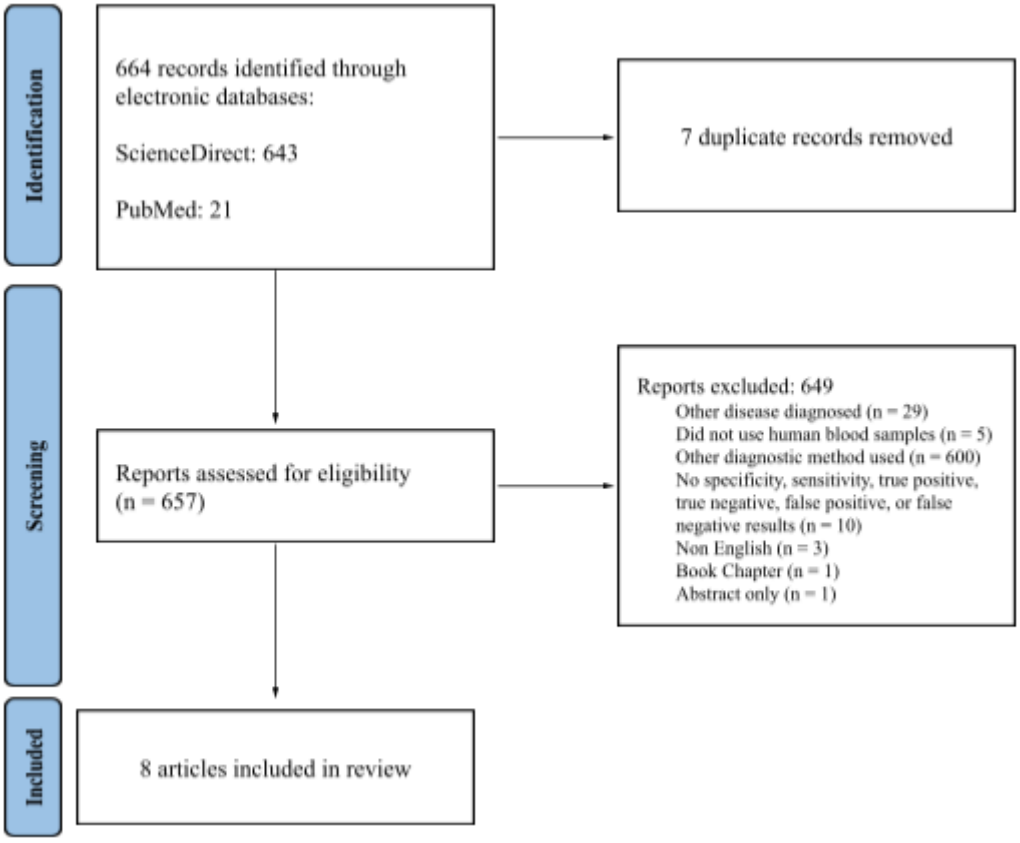

Figure 1: PRISMA Flow Diagram Presenting The Detailed Search Strategy

All articles were published in English between the year 2008 to 2022. There were four paired tests, and one internal validity, external validity, proof of concept, and immunologic study included in the review. Of which three articles were from China, two from America, and one from Japan, Germany, and the United Kingdom (Figure 2).

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Figure 2: Geographical distribution of included studies and the number of articles per country (a)USA (b)UK (c)Germany (d)China (e)Japan

Among the eight articles, four used plasma samples, while the other four studies each used whole blood and plasma, cell culture supernatant and plasma, and serum. In addition, one article did not specify the type of blood sample used. In terms of the index test, three utilized different types of RT-LAMP, namely, hydrogen ion-selective RT-LAMP, pH RT-LAMP, and variant-tolerant RT-LAMP, whereas the five others used the conventional RT-LAMP in examining the sample for HIV-1. These data are summarized in Table 2. Table 3 summarizes the outcomes of the included studies.

Table 2 General Characteristics of Included Studies

Author, Year, Setting	**Design**	**Sample Size**	**Sample**	**Index Test**	**Index Test Gene Target**	**Reference Test**
Curtis et al., 2008, USA	Experimental (Internal Validity)	10	Plasma sample, whole blood samples	RT-LAMP	Protease and p24	PCR and RT-PCR
Curtis et al. 2018, USA	Experimental (Immunologic)	61	Plasma samples	RT-LAMP	Integrase	EIA and Western Blot
Gurrala et al., 2016, England	Experimental (Proof of Concept)	991	Plasma samples	pH RT-LAMP	Integrase	VERSANT HIV-1 RNA 3.0 Assay (bDNA)
Hosaka et al., 2009,	Experimental (External	97	Plasma samples	RT-LAMP	Integrase	HIV Ab testing

Japan	Validity)					(AxSYM HIV 1/2 gO) and PCR
Kong et al., 2021, China	Comparative Accuracy (Paired test)	57	Serum samples	H^+-RT-LAMP	Not specified	RT-qPCR
Li et al., 2021, China	Comparative Accuracy (Paired test)	127	Plasma samples	Variant-tolerant RT-LAMP	Integrase	RT-qPCR
Odari et al., 2014, Germany	Comparative Accuracy (Paired test)	346	Plasma samples	RT-LAMP	Integrase	Abbott m2000rt system (RT-qPCR)
Zhao et al., 2012, China	Comparative Accuracy (Paired test)	171	Serum samples	RT-LAMP	Integrase	Western Blot and ELISA

Table 3 Results of Included Studies

Studies, year	**True Positive (TP)**	**False Negative (FN)**	**False Positive (FP)**	**True Negative (TN)**	**Total Sample**	**Sensitivity**	**Specificity**	**Limit of detection**
Curtis et al., 2008	Heat-treated Plasma Samples							100 to 1000 RNA copies/tube
	3	2	—	—	5	60%	—	
	Whole Blood Samples							
	4	1	—	—	5	80%	—	
Curtis et al. 2018	50	11	—	—	61	81.97%	—	10^4 RNA copies/mL (94.3%)
Gurrala et al., 2016	Tube assay							
	824	177	0	18	991	82.32%	100%	10 RNA copies (lowest) >1000 RNA copies/reaction (95%)

	On-chip assay							
	93	71	0	18	164	56.7%	100%	>1000 RNA copies/reaction (88.8%)
Hosaka et al., 2009	56	0	0	41	97	100%	100%	120 copies/mL
Kong et al., 2021	39	1	1	16	57	97.5%	94.1%	10 copies per tube
Li et al., 2021	39	6	0	37	82	86.67%	100%	< 18 copies per reaction (95%)
Odari et al., 2014	203	28	1	114	346	87.88%	99%	1.2×10^3 copies/mL
Zhao et al., 2012	18	0	0	153	171	100%	100%	155 copies/mL (100%)

4 DISCUSSION

With the continuous spread of HIV-1 despite the establishment of therapeutic measures, early detection of the virus—seroconversion included (Odari et al., 2014), is still significant in the prognosis, control, and prevention of the infection. In spite of prevailing conventional methods effectively detecting the peak levels of viremia and mucosal shedding of HIV-1 during acute infection, they are still posed as ineffective due to their cost, high-technical skill requirement, and sophisticated equipment. This is in particular with indigent areas and immediate care health facilities where resources are limited. The opportune introduction of RT-LAMP in the detection of HIV-1 highly suits the factors lacking in the diagnosis of HIV-1 with its rapid and cost-effective advantages. With its turnaround time of sixty (60) minutes at most, compared to the two to three (2-3) hours of RT-PCR and PCR marked a huge cut down on the intensiveness of conventional procedure. The formation of a magnesium-pyrophosphate by-product, appearing as white precipitate indicative of a positive result, enables the visualization of the reaction through turbidity. Interpretation of undetected results is enhanced under UV irradiation with Fluorescent Detection Reagent and intercalating dye or the addition of a nucleic acid stain, such as PicoGreen. Since the assay is under isothermal conditions, amplification of the RNA and/or DNA of HIV-1 does not need the aid of sophisticated equipment such as thermocyclers (Curtis et al., 2008; Hosaka et al., 2009).

The range of sensitivity of RT-LAMP situated in the lower limit of detection (1000 copies/mL) compared to that of plasma viral load that may extend from 10^3- 10^8 viral

copies/mL, will reach a detection rate of 95% (Curtis et al., 2008; Gurrala et al., 2016). Consequently, this makes it inferior to the UltraSensitive in-vitro Assay (Roche), but still superior to the Standard Assay kit with 400 copies/ml and conventional RT-PCR assays with 4-fold sensitivity (Zhao et al., 2012). Nonetheless, an increase in sensitivity is possible by increasing the volume of the overall reaction mix that elevates its detection capacity — remarkable for infection with low range viral loads (Curtis et al., 2008; Hosaka et al., 2009). Direct heating of the samples is another proposed alternative, giving a higher sensitivity than RNA extraction. In doing so, detection of proviral DNA and/or RNA which endures throughout infection warrants diagnosis of individuals beyond the primary infection (Hosaka et al., 2009). Regardless, confidence in the said remedies needs further investigation (Odari et al., 2014).

The primer in RT-LAMP accounts for six primers, including four specific primers and isothermal Bst DNA polymerase (loop primers), which equips the method to distinctively detect eight regions of the target sequence. Analysis of the agreement between seronegative samples and negative samples run under PCR showed total specificity, supporting the case (Hosaka et al., 2009). This feature promotes the high specificity of the method and the low probability of false-positive results (Hosaka et al., 2009; Zhao et al., 2012). Even so, reports of negative samples may still present non-typical amplification suspiciously caused by the self-reactivity of oligonucleotides in the reaction (Curtis et al., 2008). Thereon, the design and optimization of the primers are vital in the performance of RT-LAMP.

RT-LAMP is also presented as an ideal confirmatory test for HIV-1. Although the lacking degree of RT-LAMP in its sensitivity, specificity, reproducibility, and potential for automation detain the method to be categorized as such (Zhao et al., 2012). At the outset, primers of RT-LAMP specifically recognize the M group of HIV-1. In a study from Japan, 56 group-M HIV-1 out of 57 HIV-1 strains are detected, missing one group-O strain (Hosaka et al., 2009). Other studies share the same finding wherein a lower limit of quantification at 9.8×10^3 detects group-M samples within the value (Odari et al., 2014). Multiplexed integrase primers have recently demonstrated detection of the diverse subtypes of HIV-1 Group-M viremia while having a consistent detection rate (Curtis et al. 2018), which somehow answers the raised question about the universal use of RT-LAMP, and its detection of other subtypes.

Today, improvements in the development of the assay brought a variant-tolerant RT-LAMP technique which resolves the issue by having an 85.7% advance on the positive detection rate of the conventional RT-LAMP visualized with lateral flow strips or another colorimetric version (Li et al., 2021). Meanwhile, in a study by Gurrala et al., a novel low-buffer HIV-1 pH-LAMP was incorporated with a complementary metal-oxide-semiconductor (CMOS) chip allowing multiplex assay under one run (Gurrala et al., 2016).

With the characteristics of RT-LAMP being a rapid and easy diagnostic tool, integration of the diverse variety of HIV-1 viremia will exemplify its point-of-care and resource-constrained setting. Further exploration on the detection of RT-LAMP is still extensively needed, specifically to construct a technique that covers all subtypes including recombinant forms (CRFs) and unique recombinant forms (URFs) of the HIV-1 M group.

4.1 Limitations

The conclusions from 3 out of 8 studies were made for modified versions of the RT-LAMP, which may in turn affect the overall result of this review to assess the performance of RT-LAMP. However, this review does not analyze the differences among the modified RT-LAMPs used in the evidence. There is also a lack of standardization in the diagnostic accuracy of the studies, particularly the protocols which vary among laboratory sites. These

include the pretesting or confirmatory testing of samples, preparation of samples, type of samples used and their collection, and the RNA and DNA extraction process.

Another limitation of the evidence is the incomplete reporting of the studies. Among the eight studies, two did not directly identify the clinical specificity and sensitivity of RT-LAMP, whereas one study did not directly identify the specificity. In other studies, the clinical specificity and sensitivity of RT-LAMP were calculated using the samples with amplifications below the limit of detection reported, possibly causing low sensitivity results. Other factors for the low sensitivity reported in the studies are the various technologies used and the possibility of the new RT-LAMP assays not being established well enough during their publication. Furthermore, the sample sizes of the studies varied from one another, some having as low as five while others had as high as 991 samples.

Systematic reviews have no minimum number of studies required, but due to the overall low number of studies included in this review, further research is desired to have a better unanimity from the collected and analyzed data. Additionally, a meta-analysis of the pooled data was not performed due to time constraints. Thus, the quantitative analysis of the diagnostic value of RT-LAMP in the diagnosis of HIV-1 is not available.

4.2 Implications of the study

This systematic review aims to investigate the RT-LAMP's performance in diagnosing HIV-1 based on its sensitivity and specificity. It was accomplished by including relevant studies that involved the specificity and sensitivity in detecting the presence of HIV-1 in human blood samples using RT-LAMP. Consequently, a practical contribution of the results of this systematic review is that the RT-LAMP is not yet suitable to be used as a diagnostic tool for HIV-1 despite the given knowledge that the technique is cost-effective and simple. The sensitivity results of RT-LAMP for HIV-1 as a diagnostic tool shows that its chance of being used for clinical purposes is not yet plausible. With this paper's results, the medical field will be provided accessible evidence on the diagnostic performance of RT-LAMP and may allow improvements to this alternative diagnostic method for HIV-1. It is believed that this systematic review is timely in both the general public and medical community, given that roughly 84% of HIV-infected people around the globe were aware of their HIV status in 2020, and there is still a remaining 16%, approximately 6 million people, who didn't have access to HIV testing services (“The Global HIV/AIDS Epidemic,” 2021).

For future systematic reviews, the inclusion and utilization of more journal articles will be necessary to refine and further strengthen the results. The paper could be improved as discussed in the limitations of the study in the area of studies that should be included. This is necessary to have a better foundation for the results and a more credible output. Moreover, a meta-analysis of the aforementioned journal articles is recommended to provide quantitative analysis.

5 CONCLUSION

A systematic review of eight studies showed inconsistency in the diagnostic value for RT-LAMP, as seen in its sensitivity values ranging from 53.85% to 100% and specificity values ranging from 94.1% to 100%. However, the RT-LAMP technique's short turnaround time, ease of use, and cost-effectiveness make the assay a promising tool for diagnosing HIV-1. Thus, the authors conclude that RT-LAMP might not yet be a valuable tool in diagnosing HIV-1. However, several limitations, including the credibility of the studies, differing sample sizes and units reported, the inclusion of novel RT-LAMP assays, and the absence of a meta-

analysis of the pooled data, leave room for further improvement in future studies and reviews on the same topic.

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