Review Article

Using Saliva and LAMP for Non-invasive Detection of Communicable Pathogens in Low Medical Access Regions

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Abstract

Diagnosis of pathogens using invasive sample collection (blood, nasopharyngeal) and expensive equipment is slow to implement in a pandemic setting. Saliva is an under-utilized matrix that has a lot of potential to be utilized for diagnosing communicable diseases. This is a suitable matrix in places with low medical access or where the infrastructure is lacking due to an outbreak. With a non-invasive matrix it allows for faster sampling and reduces the amount of exposure of the disease to others including medical professionals. New molecular biology techniques can allow the capture and amplification of small amounts of viral DNA/RNA, bacterial DNA, or parasitic DNA. Several different techniques and tools could be used to amplify and diagnose viz. Polymerase Chain Reaction, Isothermal Loop-Mediated Amplification (LAMP). Recently, CRISPR-Cas, optimized Sanger sequencing and Next Generation Sequencing have been developed but it is not useful for routine diagnosis. For low access regions or where diagnostic testing infrastructure is lacking LAMP is clearly optimal due to its limited need for equipment and reagents. Collection of saliva is the best biofluid for low medical access regions. In this review we show that LAMP can be utilized to diagnose several diseases from a simple saliva sample in different regions of the world with low medical access. In this review we will look at specific pathogens and suggest using LAMP to diagnose from genomic material found in saliva.

1. Introduction

1.1 Problems with Detecting Pathogens using Modern Diagnostic Approaches

The casualties of the COVID-19 pandemic have highlighted the importance of rapid, non-invasive, affordable diagnostics in our society. Testing for infection not only has significantly reduced COVID-19-related deaths (estimated one death per 940 to 8,838 tests) but has also eased economic pressure and diminished the need for strict lockdown measures in many regions [1]. Nasopharyngeal swabs were used over non-invasive matrixes like saliva despite existing evidence of significant viral load in saliva [2]. Viral tests, including nucleic acid amplification tests (NAAT) and antigen tests have been essential for detecting an active pathogen, particularly in the absence of clinical symptoms. A considerable proportion of the spread of COVID-19 has been attributed to pre-symptomatic individuals (those who are infectious but do not yet present symptoms) and asymptomatic individuals (those who are contagious and who will never present symptoms), who, without a diagnosis, can unknowingly spread the virus to those around them [3]. While high-income countries (HICs) benefited from the emergence of fast diagnostics for COVID-19, many low- and middle-income countries (LMICs) have been unable to

access these tools sufficiently. Numerous reasons account for this, including a high cost, poor usability of the products, and manufacturing capacity [4]. The global humanitarian organization: Médecins Sans Frontières has described that there are clear negative consequences for the population health in areas where diagnostics are not readily accessible [5]. These diagnostic gaps between countries extend far beyond COVID-19, and it continues to be a problem for many deadly diseases such as tuberculosis, HIV, and malaria. Although considerable barriers exist further down the pipeline, developing rapid and cheap diagnostic tests for infectious pathogens can significantly ease the financial burden of manufacturing and distribution.

1.2 Saliva as a Matrix for LAMP

Accessibility for diagnostics is both dependent on the method of analysis as well as the method of sampling. Various labs have shown LAMP to be an effective method of disease detection via blood sampling or nasopharyngeal swabs [6]–[9] (*Figure 1*). However, this type of sampling requires trained professionals with proper laboratory equipment to ensure the patient's safety. Individuals may also be reluctant to provide a blood sample, given its invasive nature. Saliva, on the other hand, is a much simpler matrix and has advantages over using blood, viz. convenience in the collection, lower cost, patient acceptability, and less sample processing[10]–[15]. However, the main disadvantage of using saliva is that there can be a lower viral or bacterial load for specific diseases than blood. New collection methods, better purification techniques, better dyes, and more detectors that are sensitive can increase the reliability of using saliva to obtain a small number of copies.

A thorough review of the literature supports the potential for using LAMP to detect various pathogens in saliva. We sorted the pathogens into four categories: sexually transmitted infectious (STIDs), respiratory diseases, childhood diseases, and tropical diseases (*Figure 1, lower panel*). This empirical evidence can direct more efforts towards optimizing LAMP diagnostics in saliva to detect infectious diseases better worldwide.

1.3 Loop-mediated isothermal amplification (LAMP).

LAMP is a simple, cheap, and rapid method of NAAT that has shown great potential for diagnostics (*Figure 2*). It requires only four to six primers, a DNA polymerase, and a laboratory water bath or regulated heat block [16] [17]. Under isothermal conditions, the cyclic production of self-hybridizing loop structures can produce upwards of 109 copies of the target DNA in under an hour and at the same temperature so that it can be used as a high throughput tool [18]. LAMP can also use to detect target RNA sequences with the addition of reverse transcriptase. These characteristics make LAMP an excellent alternative to polymerase Chain Reaction (PCR), often the standard method for disease detection. With multiple primers, the specificity of the reaction is much higher in detecting six or eight regions of DNA as opposed to two in PCR.

Furthermore, studies have shown that LAMP can be 100 times more sensitive than the standard PCR method[19], [20]. Combining this detection with spectroscopy, turbidity, or colorimetry, the latter two of which can be observed with the naked eye [21][22]. Recently, researchers have started to overcome the LAMP assay's major disadvantage, its inability to multiplex. Several reports are shown that techniques can be used to multiplex LAMP to broaden its detection range [23]. Overall, the specificity and sensitivity of the LAMP detection are superior to a polymerase chain reaction (PCR), the current gold standard method for infectious disease diagnosis.

Additionally, LAMP requires less training, equipment, and money. As a result, LAMP has recently been implemented to detect many infectious diseases [4], [23]–[25]. Depending on the condition, the most common matrices collected in LAMP are from a venous blood draw or capillary fingerpick, nasopharyngeal swabs, urine or sputum collection [24] [26], [27].

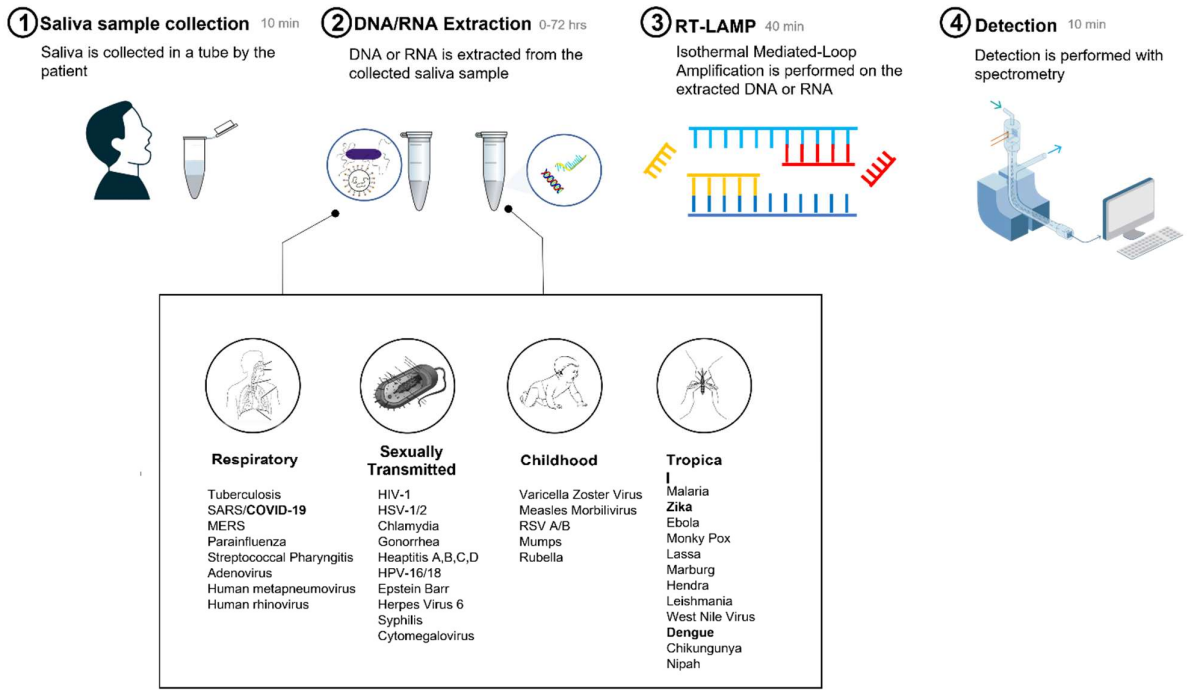

Figure 1 - Overview of application LAMP for detecting various pathogens in saliva. 1) Saliva is collected by spitting in a tube and can be performed at home by the patient or in a clinic. **2)** Pathogenic nucleic acids can be processed from saliva with basic equipment, where the DNA or RNA from the desired pathogens are extracted. The parasitic, viral and bacterial pathogens are categorized into four main groups: respiratory, sexually transmitted, childhood and tropical pathogens. **3)** Amplification of nucleic acid through LAMP are performed on the extracted DNA/RNA from the sample. **4)** The detection using spectroscopy, colorimetry or turbidity can be performed. Overall, the process is quicker than conventional PCR or ELISA testing for detection of a pathogen.

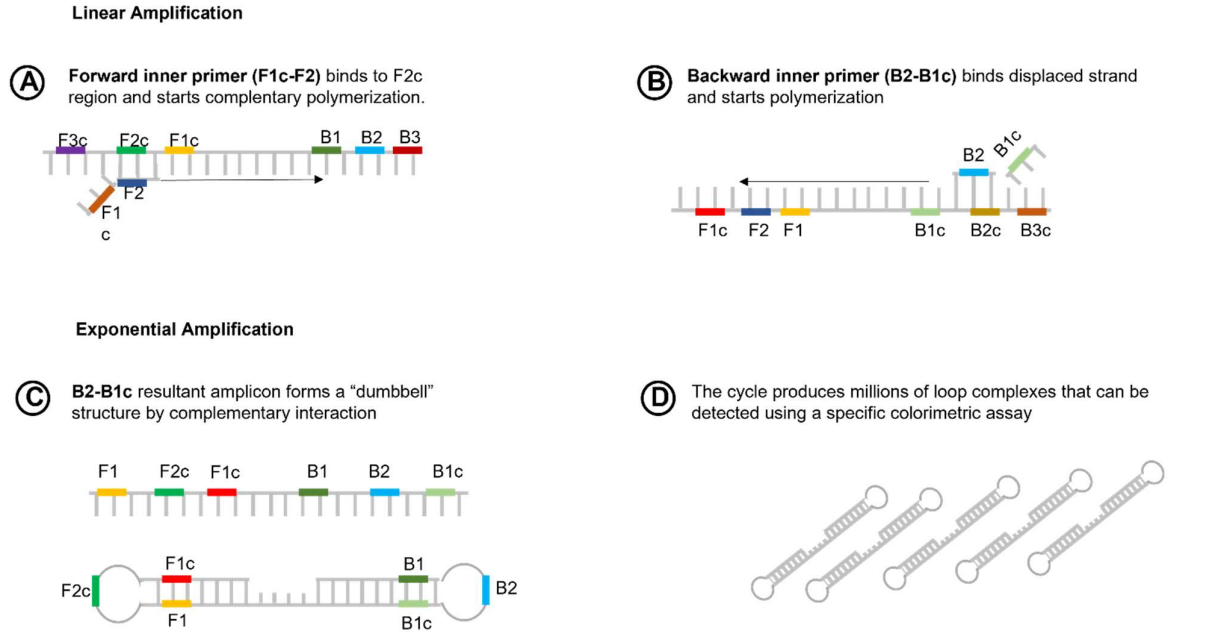

Figure 2 - LAMP uses 4-6 Primers which are designed to bind to specific DNA or RNA templates. **A&B)** The LAMP is initiated in a linear non-cycling amplification by nicking and displacing the DNA strand or binding to the RNA strands in the forward direction and then the backwards direction. **C)** An overhang is produced in both directions of the F2 and B2 regions which makes a dumbbell like structure. **D)** This is exponentially increased in new dumbbell like structures which is further amplified into complementary structures with loop primers. Due to the small size of these dumbbell structures, they can be copied faster. Intercalating fluorescent dyes can be used to detect with spectroscopy. Additionally, a colorimetric or turbidimetric change can be observed using pH sensitive dyes that change color with increasing cycles.

2. Load in Saliva of Different Diseases and Use of Saliva with LAMP

2.1 Sexually Transmitted Infections

It is estimated that nearly one million people worldwide are infected with one of the four curable sexually transmitted infection (STI) everyday [28]. Conditions can result in various consequences, from minor clinical symptoms to infertility. While only a few STIs can be transmitted through saliva, it is becoming increasingly clear that STIs transmissible by other means is still detectable in saliva. Herpes (HSV-1, Epstein Barr, Cytomegalovirus), HIV-1 (Human Immunodeficiency Virus), Hepatitis A-D, and bacterial infections, Syphilis, and Chlamydia can be detected in saliva in specific quantities, which can be subsequently seen using LAMP. Here we will focus on two prevalent diseases, HSV-1 and HIV, where collecting saliva and utilizing LAMP could be the best way to diagnose.

Herpes simplex virus 1 (HSV-1), primarily transmitted via oral contact, infects around 67% of all individuals under the age of 50 [29]. Most infections are asymptomatic, and "shedding" has been identified as the primary reason for rapid spread. This can be detected in saliva in quantities of $1x10^2$-2.810^6copies/mL [30], which is well above the LOD needed for LAMP (*Table 1*). Although no cure currently exists, antiviral treatments are available to help relieve symptoms and reduce the risk of spreading during the most contagious time points of the infection. Kaufman et al. [31] surveyed healthy adults and discovered that 98% of volunteers experienced some level of HSV-1 shedding during a 30-day period. A simple non-invasive test to know whether a person has it or is shedding HSV-1 can be used to reduce the spread and, as a companion, diagnostic to observe the reduction of symptoms.

In a study from 2010, thirty-six out of forty-seven HIV-infected participants had a detectable level of HIV-1 RNA in saliva by RT-PCR (3). The salivary viral load was also correlated with plasma load, indicating that saliva could be a reliable detection matrix during the early stages of infection when the virus is rapidly replicating. Current RT-LAMP for HIV-1 RNA detection in whole blood has a LOD of 1700 copies in a 10 µL sample, while the median RNA level in infected volunteers was 75 copies/mL [32]. With further optimization, this assay has the potential to detect HIV-

1 RNA in most infected individuals. Rapid, early detection would help reduce the further spread of HIV and prevent the progression to the life-threatening acquired immunodeficiency syndrome (AIDS).

Table 1 – **Sexually Transmitted diseases**

Disease	Biomarker	Biomarker Concentration in Saliva	LOD	Reference
HIV	**RNA**	Median: 75 copies/ml, Range: 52 - 141,621 copies/ml	DNA: 10^2 copies/mL, RNA: 10^5–10^4 copies/mL	[32]–[34]
HSV-1	**DNA**	34% of positive individuals had 240 to 24,000 copies/10 µL, 32% had >24,000 copies/10 µL *study performed in asymptomatic adults	DNA: 10 copies/µL	[20], [31], [35]
CHLAMYDIA (*Chlamydia trachomatis*)	**DNA**	Median bacterial load of chlamydia was 446 copies/ml (IQR: 204-1390 copies/ml) in saliva (from men + with oropharyngeal chlamydia)	DNA: 11.25 copies (3µL sample is smallest)	[36], [37]
GONORRHEA (*Neisseria gonorrhoeae*)	**DNA**	Median load: Culture positive, 2.2 × 105 copies/ml; culture negative, 2.7 × 105 copies/ml	1 pg/µL (1 × 103 CFU/mL cells)	[38], [39]
HAV (Hepatitis A virus)	**RNA**	9.3 × 102 to 1.9 × 104 copies/ml	0.4 - 0.8 FFU / 5 µL (depending on virus strain)	[14], [40]
HBV (Hepatitis B virus)	**DNA**	Median HBV DNA levels in saliva: 2.27 × 104 geq/ml, range: 373 geq/ml to 9.25 × 106 geq/ml	0.002218 pg/µL	[8], [41]
HCV (Hepatitis C virus)	**RNA**	Salivary HCV RNA prevalence was 52.4% in one study, 3.32 log10 copies (2.1 x 103 copies/mL) in the saliva in another	10 copies/mL	[42], [43]
HPV (Human Papilloma Virus)-16	**DNA**	HPV-16 DNA viral load in saliva increased exponentially across the 36-month follow-up period (from 3.43 to 1,281.69 copies/50 ng) and subsequently declined to undetectable levels post-tonsillectomy (case study)	LAMP-LFD: 10^1 plasmid copies, LAMP-turbidity: 10^3 plasmid copies	[44], [45]
HPV-18	**DNA**	Detectable in 5mL saliva samples	LAMP-LFD: 10^0 plasmid copies, LAMP-turbidity: 10^1 plasmid copies	[45]
HSV-2	**DNA**	Oral shedding of HSV-2 is infrequent, of 1388 participants, 44 (3.2%) had HSV-2 isolated from the mouth on at least one occasion	LAMP (agarose gel electrophoresis): 1,000 copies/tube, LAMP-turbidity: 10,000	[46], [47]

			copies/tube (5 µL sample)	
EPSTEIN BARR	**DNA**	Study in mothers/children in Africa (asymptomatic), children: detected DNA in 90% of the children, median [interquartile range {IQR}], 5.2 [4.2–6.0] vs. mothers: 4.8 [3.7–5.6] log10 copies/mL of saliva	100 copies of plasmid (both electrophoresis and turbidity methods), or 5 copies / sample tube with heat treatment (see notes)	[7], [48]
HSV-6	**DNA**	Majority of the samples contained between 10^4 and 10^ 5 copies/mL, with a median value of 23,870, samples that underwent freeze-thaw cycle had median of 3,497 copies/mL	Original HHV-6 LAMP: 50 copies/tube, raised concentration of each primer: 25 copies/tube	[49], [50]
SYPHILIS (*Treponema pallidum*)	**DNA**	All cases: median (range) loads of *Tpp47* and *pol A* in saliva were 627 (0–101,200) copies/mL and 726 (0–117 260) copies/mL, respectively; primary syphilis: between 0-2,000 copies/mL for both *Tpp47* and *polA*	5.4 × 10−6 ng/µL	[51], [52]
CMV (Cytomegalovirus)	**DNA**	Range: from 7.5x10^2 to 8.2x10^9 IU/ml saliva (median: 9.3x10^7) or 1.5x10^2 to 5.6x10^10 IU/10^5 cell equivalents (median: 3.5x10^6) (data from congenital CMV in newborns)	10 copies/µL	[53], [54]

2.2 Respiratory Diseases

Infections of the respiratory tract caused by viruses, bacteria, fungi, and parasites are a significant component of morbidities and mortalities worldwide [1], [3][1],[2]. Epidemics and pandemics over the past decades have resulted in growing attention to the detection of respiratory diseases, where several outbreaks have occurred in the last 30 years [1],[2]. From tuberculosis outbreaks in the 1990s [3], to severe acute respiratory syndrome (SARS) in 2002[4], followed by influenza A in 2009, to the most recent outbreak of the novel COVID-19 virus in 2019 [1],[2]. Respiratory diseases like influenza, COVID-19, rhinovirus, and adenovirus can all be easily detected in saliva in high quantities and have all been documented to be detected with the LAMP method (*Table 2*). In the following section, we will focus on reviewing the recent research on the use of LAMP on saliva samples for the detection of tuberculosis, influenza, and coronavirus, as these three are more deadly respiratory diseases in the world today.

Tuberculosis **(TB)** are among the leading causes of morbidity and mortality in developing countries [55] . TB is an airborne disease caused by *Mycobacterium tuberculosis* (MTB), which primarily infects the lungs. The active disease is characterized by chronic choughs, chest pain, fevers, weakness, and weight loss [55]. The failure of TB incidence control in developing countries is owing to the inadequacy of rapid and accurate point-of-care diagnostics that can be used in resource-limited settings [4], [5][. A research study conducted by Kumar et al. [8] have demonstrated the high sensitivity and specificity of a LAMP-based assay for the detection of TB [19] . The LAMP assay exhibited a limit of detection of 5 fg, while analysis with mPCR showed a detection limit of 5 x 10-5 ng (corresponding to 50 fg) of DNA, which makes the LAMP assay ten times more sensitive than mPCR [19]. Another recent study from 2019 performed by Shete et al.[24] also assessed the diagnostic accuracy of TB-LAMP as an alternative to sputum smear microscopy [24]. The study demonstrated a higher sensitivity of the TB-LAMP assay compared to the smear microscopy but with similar specificity [24]. One aspect of TB, however, is that the disease needs to be in its active form to be detected. Thus, only the active form might have enough load to be used.

The bacterial DNA concentration of MTB in saliva has not been identified in literature yet, resulting in limited knowledge of implementing saliva as a detection matrix in TB. However, a study from 2017 performed by Namuganga et al. [56] evaluated the suitability of saliva for TB diagnosis through a complex of 10 biomarkers [56]. The study demonstrated a significant difference in detection levels between saliva and serum, showing that some biomarkers had a higher presence in saliva than in serum and vice versa. The concluding remarks of this study pointed toward the worth of further research on TB biomarkers in saliva. Therefore, it would serve as valuable data to further investigate the detection of bacterial DNA in the saliva of TB patients to identify if the viral loads are high enough to be detected.

Coronaviruses **(CoVs)** belongs to a family of highly pathogenic single-stranded RNA viruses that mainly cause respiratory- as well as neurological and intestinal infections in humans and animals [1], [57]. Over the past decade, highly pathogenic coronaviruses have been emerging and re-emerging. This has led to epidemics and pandemics, which have challenged the public health system worldwide [4]. So far, the most pathogenic coronavirus strains identified are SARS-CoV, MERS-CoV, and SARS-CoV-2. These strains are more susceptible to causing lower respiratory tract infections, leading to a more severe disease course like acute lung injury, septic shock, and multi-organ failure [1], [57], [58] .

Clinical application studies have evaluated the use of LAMP to detect pathogenic coronaviruses. Hong et al. [12] have developed and validated the use of RT-LAMP to detect SARS-CoV [59]. The RT-LAMP assay demonstrated the detection of SARS-CoV RNA with a sensitivity and specificity of 100% and 87%, respectively. This showed a 100-fold higher sensitivity than the conventional RT-PCR method and a detection limit of 0.01 PFU [59], [60]. The application of RT-LAMP has also been assessed for detecting MERS-CoV and was reported by Shirato et al. [61]. The research group demonstrated that the primer sets targeting the conserved nucleocapsid protein region can detect the MERS-CoV virus with equivalent sensitivity to the RT-PCR and with a limit of 3.4 copies of MERS-CoV RNA. Furthermore, the RT-LAMP also exhibited high specificity for the MERS-CoV virus, showing no cross-reactivity to other respiratory viruses [61]. The promising prospects of RT-LAMP application for SARS-CoV-2 detection have also been investigated by the research group Jiang et al. [15]. The group assessed the RT-LAMP for the diagnosis of COVID-19 disease as a comparison to the use of RT-PCR. Jiang et al.[24], [62]concluded that the analytical specificity and sensitivity of the RT-LAMP assay was 100% with a determined LOD between 500-1000 copies/ml of the virus. Another study performed by Garneret et al.[63] also demonstrated a LOD of 1 genome copy/ul (1000 copies/ml) when the performance of RT-LAMP was compared to RT-PCR (*Table 2*) [63].

The current diagnostic testing matrices for coronavirus infections rely on naso- and oropharyngeal swabs [64]. However, saliva as a sampling matrix has been increasingly evaluated for detecting coronaviruses with the emergence of the novel COVID-19. Compared with the swab-based testing methods that include several limitations like the discomfort of patients and the need

for skilled personnel, saliva is increasingly being suggested and assessed for detection of coronavirus infections [64], [65]. A current research study by Zhu et al. [66] further evaluated the clinical performance of saliva in comparison to respiratory tract specimens. The research group demonstrated salivary viral loads of 104 - 108 copies/ml during the first week of symptoms and that these values were comparable to sputum and throat swabs [19]. Detection of SARS-CoV in saliva has also been demonstrated as a reliable sampling matrix by Wang et al.[67] , as the group showed that SARS-CoV RNA could be detected in saliva between 7.08x103 – 6.38x108 copies/ml [11], [67].
With the comparison between the salivary viral loads of COVID-19 and SARS and the reported detection limits of the RT-LAMP, the currently developed assays demonstrate considerable potential in the detection of coronavirus infections in saliva [68]. No studies have been performed on detecting RNA concentration in saliva for viral infection with MERS-CoV. Therefore, limited data are found on the potential of saliva as a sampling matrix. However, a recent study from 2019 performed by Hemida et al. [68] detected MERS-CoV RNA in collected saliva samples from dromedary camels. Collectively, the results from Hemida et al. and the results obtained for COVID-19 and SARS-CoV indicate the possibility of using saliva as a sampling matrix for MERS-CoV detection [68]. The conclusions suggested in these studies should provide future investigations to validate the proposed findings in these studies.

Influenza has over the last 100 years caused several widespread outbreaks. These outbreaks range from 1918 with the Spanish flu (H1N1), 1957 with the Asian flu (H2N2), 1968 with the Hong Kong influenza (H3N2) to the outbreaks with swine influenza (H1N1) and Influenza A (H3N2) in 2009 and 2014, respectively [23]. Several diagnostic methods are currently used to detect influenza virus infections, like viral cultures, RT-PCR tests, and antibody-based methods like ELISA [1]. Using saliva in LAMP would be beneficial for diagnosing new forms of the flu virus but with less cost. The LAMP approach to detecting influenza viruses has been developed and assessed by several research groups [24], [25]. Shigemoto et al.[69][24] have reported the detection of influenza A subtypes H1N1 and H3N1 with RT-LAMP, with the absence of cross-reaction occurrence between the influenza A subtypes and other respiratory diseases [24]. The use of RT-LAMP was further demonstrated by Jeong Ahn et al.[25] [70] that developed an RT-LAMP detection system for both influenza B and influenza A H1N1, H3N2, H5N1, H5N6, H5N8, and H7N9 subtypes . The developed RT-LAMP assay showed a high sensitivity ranging from 0.1-100 viral genome copies for the different influenza subtypes (see table 2), showing a higher sensitivity than one-step RT-PCR (92.3 vs. 98.9%)[70] [25]. Evaluation of saliva as a diagnostic matrix for influenza detection has also been conducted for the influenza B and influenza A (H1N1) viruses [27]. A study from 2016 performed by the research group Sueki et al. [27] demonstrated the utility of saliva as an alternative diagnostic matrix to nasopharyngeal swabs. The study's results showed a concordance between the nasopharyngeal swabs and saliva of 95.8%, with a viral load of more than $1x10^2$ copies/µl of the influenza gene (Table 2) [27]. The detection of influenza virus Type B and H1N1 influenza A have been well demonstrated in saliva compared to other test specimens. However, despite the potential for diagnosis of influenza in saliva, no further data have been obtained to support the current findings of the salivary loads of the other strains. Therefore, salivary viral loads for influenza viruses need to be evaluated in the future for more substantiating data.

LAMP assays have also been developed and evaluated for the diagnosis of other respiratory infectious pathogens like parainfluenza viruses (PIV), human rhinovirus (HRV), human adenovirus (HAdV) and for Human metapneumovirus (hMPV) [27–30][27–30]. Current detection methods for the mentioned diseases relies on antigen-based assays like ELISA or RT-PCR [75]. However, the application of LAMP assays has been exploited for the detection of six common respiratory viruses among others PIV, HRV and HAdV by several research groups [71]. The sensitivity of the developed RT-LAMP assays was evaluated through viral RNA and demonstrated a limit of detection of 1×10^2 copies/ml for both PIV, HRV and HAdV [71]. A research study conducted by Song *et al.* [73] also showed the successful detection of both hMPV A and B with an RNA copy number of 4.33×10^{10} copies/µL and 4.53×10^{10} copies/µL, respectively [72], [73].

The detection of PIV, HRV and HAdV have also been assessed in a comparative study between saliva and nasopharyngeal swab specimens by Kim *et al.* [72]. The results of the study showed a comparable detection rate from saliva and nasopharyngeal swab specimens when assessed by RT-PCT. hMPV has also been demonstrated to be detected in saliva samples when compared to the traditional nasopharyngeal specimens by KW To *et al.* [74]. The overall results observed so far for these respiratory diseases, indicates that saliva may be a viable alternative for nasopharyngeal swabs for all these diseases [72]

Table 2 - Respiratory diseases

Disease	Biomarker	Biomarker Concentration in Saliva	LOD	Reference
Tuberculosis	DNA	-	5 fg/MTB DNA	[76]
COVID-19	RNA	10^4 - 10^8 copies/ml	1 genome copy/µl 500 – 1000 copies/ml	[77]
MERS	RNA	-	3.4 copies/RNA [78]	
SARS	RNA	7.08×10^3 - 6.38×10^8 copies/ml	0.1 PFU	[79]
Parainfluenza	RNA	-	$1x10^2$ copies/ml	[71]
Influenza B	RNA	10^2–10^6 copies/µl	10^0 - 10^5 copies/µl	[80]
Influenza A (H1N1)	RNA	10^2–10^6 copies/µl	10^{-1} - 10^4 copies/µl	[26][26]
H3N2 H5N1 H5N6 H5N8 H7N9	RNA	-	10^{-1} - 10^4 copies/µl 1 x 10^2 copies/ml	[71]
H9N2	RNA	-	10 copies/reaction	[82]
Adenovirus	DNA	-	1 x 10^2 copies/ml	[71]
Human metapneumovirus A	RNA	-	4.33×10^{10} copies/µl	[73]
Human metapneumovirus B	RNA	-	4.53×10^{10} copies/µl	[29]
Human Rhinovirus	RNA	-	$1x10^2$ copies/ml	[71]

2.3 Childhood Diseases

Several childhood diseases in the Western world, like VZV, Measles, Mumps and Rubella have effective vaccines against them, but are not applied worldwide [83], [84] [84]. Together with importations from other countries and the vaccine hesitancy in recent years has led to lower herd immunity which has increased the incidences of childhood infectious diseases in the west [31],[32]. The increased frequency of childhood diseases has led to a renewed awareness as well as the interest in the early detection of these diseases [31],[33]. For children testing would be a lot less invasive than using other techniques like nasal oropharyngeal swabs, or blood tests. For many of these diseases, having the testing at home with saliva samples would help reduce the spread. This is especially true for both measles and VZV where a high R0 exists to be 12-18 and 10-12 which are both more transmissible than COVID-19 [33],[34]. With the combined diagnostic platform of LAMP and saliva it would be an accurate and non-invasive method for the early detection of infectious childhood diseases (Table 3).

***Varicella zoster virus* (VZV)** is an Alpha-herpes-virus with a double-stranded DNA genome that can result in the highly contagious disease Chickenpox [88]. Following the primary infection that leads to the establishment of chickenpox, the virus enters a latency period in ganglionic neurons, which later can be reactivated to cause neurological complications [88].

VZV is highly contagious and is usually transmitted through the air to establish itself in the oral pharynx. In addition to this route, the virus is transmitted from the skin, where the VSV is shed through epithelial cells [89], [90]. The spread is mainly observed in high temperate countries, where transmission rate is highly observed among children. However, most older children and adults accommodate latent VZV that later can be re-activated and replicated [20], [89].

Current standard molecular methods for detection of VZS include dot blotting, in situ hybridization, and PCR as the standard gold method. However, the LAMP application has also been shown by Kaneko and colleagues to detect 8 VZV strains [20]. The research group demonstrated the detection of VZV with novel designed primer sets and with a 10-fold higher sensitivity than the PCR assay. Okamoto et al. also successfully detected VZV using LAMP primers targeting the VZV DNA [91]. The sensitivity of VZV LAMP was confirmed by agarose gel electrophoresis and turbidity assays, showing a detection limit of 500 copies/tube and 1000 copies/tube, respectively [91].

Studies have also been conducted to assess saliva as a testing matrix which has confirmed the detection of VZV in saliva [40], [41]. Nagel et al. [92] analyzed saliva samples obtained from three patient groups and assessed these samples by PCR. The study showed that VZV DNA could be extracted and amplified from the saliva samples and a PCR detection limit of 10 copies/ml [40]. Another study by Mehta et al. [41] also assessed the presence of VZV in saliva by PCR analysis. It demonstrated a detection limit between $1.6 \times 10^1 – 5.5 \times 10^3$ copies of VZV DNA per ml saliva [90](A55).

Respiratory Syncytial Virus (RSV) is a single-stranded RNA virus that belongs to the family of Paramyxoviridae [1]. The RSV can be classified into subgroups A and B based on the virus's membrane G protein sequence, which plays a vital role in the induction of protective antibodies and host immunity modulation [1], [93]. RSV is one of the leading pathogenic agents that causes severe upper and lower respiratory infections, with significant morbidity and mortality worldwide [93], [94]. The virus, mainly affecting infants and the elderly population, is estimated to be responsible for around 120,000 deaths annually among children under the age of 5 years and up to 3 million hospitalizations. Furthermore, RSV is the leading cause of death globally among respiratory infections in infants under the age of 1 year (A66), emphasizing the need for an accurate and non-invasive diagnosis to initiate early treatment [93], [94].

Currently, the state-of-the-art approach to identifying RSV is through PCR, ELISA, and immunofluorescence assays [1]; however, the use of RT-LAMP has also been investigated to detect RSV[61] [95]. Chen et al. developed the primer sets for RT-LAMP specific for the two subgroups A and B of RSV and evaluated the assay with commercial RT-PCR[95]. The groups successfully demonstrated the precise detection of RSV A and B with a detection limit of 1 x 102 copies/ml of RSV RNA. This result of the detection ability of RT-LAMP was comparable to RT-PCR, which showed a rate of sensitivity and specificity of 100%. Another research group also successfully detected RSV A and B [14]. Mahony et al. [95] also developed and compared the primer sets of RSV A and B in an RT-LAMP assay and a commercially available enzyme immunoassay (EIA). The study showed a higher sensitivity of RT-LAMP than the EIA assay, with a detection limit of 107 copies/5 ul of template [95].

Regarding the detection of RSV in saliva, no studies were found on the biomarker concentration of RNA in saliva. However, several studies have compared nasopharyngeal-throat and saliva swabs for detecting RSV [28], [45]. The comparison study conducted by Kim et al. evaluated the detection of RSV in nasopharyngeal and saliva swabs by RT-PCR [96]. The study demonstrated that the overall detection rate from nasopharyngeal- and saliva swabs was comparable. In contrast, the analysis performed by Robinson et al. [96] showed that throat and saliva specimens are inferior to nasopharyngeal swabs and that showed that throat- and saliva swabs were comparable when analyzed with the NAT test [96]. The studies strongly suggest that viral infections are detectable in saliva and that it would be valuable to further investigate the detection of RSV in saliva with LAMP.

Measles virus **(MeV)** is a single-stranded RNA virus that belongs to the genus Morbillivirus and causes measles, a highly contagious infectious disease primarily observed in children [97], [98]. Some of the characteristics of the disease include high fever, coughing, and conjunctivitis with subsequent rash formation [46]. MeV is often transmitted through respiratory droplets when coughing, sneezing, or saliva and displays a high transmission rate compared to other viruses [46]. Annual measles outbreaks are still observed worldwide, resulting in high mortality rates among infants in developing countries despite the presence/availability of the measles-, mumps-, and rubella vaccine[99], [100].

The diagnosis of MeV infection is assessed through serological examinations or, more traditionally, by RT-PCR [48]. But due to the limitations of RT-PCR regarding the apparatus and environmental settings in developing countries, Fujimo et al. [101] assessed the use of LAMP assay for the detection of MeV. The study successfully detected the genomic MeV RNA with higher sensitivity than RT-PCR. The LAMP assay demonstrated a limit of detection between 30-100 copies/sample within 60 min after RNA extraction. The detection of MeV in non-invasive samples like saliva

has also been validated and compared with nasopharyngeal secretions by Hutse and colleagues [46]. The research group validated the RT-PCR detection of MeV using oral fluids and showed 100% sensitivity and specificity[97] . The RT-PCR result also showed a MeV RNA concentration of 4 IU/ml, indicating the potential of oral fluids for molecular diagnostics.

Table 3 - Childhood Diseases

Disease	Biomarker	Biomarker Concentration in Saliva	LOD	Reference
Varicella zoster virus (VZV) (Chicken Pox)	DNA	$1.6 \times 10^1 – 5.5 \times 10^3$ copies/ml	500-1000 copies/tube	[36],[40]
Measles Morbillivirus (MeV)	RNA	4 IU/ml	30–100 copies/sample	[104]
Mumps (MuV)	RNA	10 copies/SH RNA	0.12 PFU	[105]
Rubella	RNA	2 copies	1 -100 PFU	[106]
Respiratory Syncytial Virus (RSV A)	RNA	-	10^7 copies/ 5 µl RNA [107] 1×10^2 copies/ml	[71]
Respiratory Syncytial Virus (RSV B)	RNA	-	10^7 copies/ 5 µl RNA [107] 1×10^2 copies/ml	[71]

2.4 Tropical Diseases

Although tropical diseases are more prevalent in Southern countries in Asia, South America, or Africa, many tropical diseases have come to Europe and North America due to the migration of people, animals, or insects. With global warming, many of these diseases represent a global threat as there is an increased opportunity for these diseases to be prevalent in places that are currently relatively cold. Thus, tropical diseases create a global problem that needs quick, non-invasive tests. LAMP has been utilized in all the disorders below (Table 4). Saliva and LAMP for Dengue, Zika, Chikungunya, and Malaria have recently been used[108], [109]. Other interventions like an mRNA vaccine are being tested for Zika and Chikungunya [110], [111]. A vaccine for Dengue was recently approved [112], and there is a long way to go for the malaria vaccine to be developed [113]. Although these infections still pose a valid threat, the abovementioned interventions can mitigate these. Thus, within this review of tropical diseases, we will investigate two pressing conditions that had recent outbreaks in low countries, Lassa and Nipah virus [114]. Utilizing saliva collection in combination with LAMP for testing rather than the more common blood draw could save a lot of time and money.

Lassa fever is endemic in parts of West Africa viz Liberia, Guinea, Nigeria, and Sierra Leone, with many neighboring countries like Burkina Fasso, Cote d'Ivoire, Togo, Benin, and Ghana also having risk. The multimammate rat spreads is an animal vector for this zoonotic disease. Crude estimates for people having the virus range from 100,000-300,000 people acquire this infection every year. It is estimated that a higher mortality rate than coronavirus through a viral hemorrhagic fever with a case mortality rate of 30%. One key aspect is that there is little surveillance of the diseases. Likely, this is because it affects some of the poorer parts of Africa. More disease management is needed to estimate how far this virus has spread and install active control measures. However, it is endemic to Western African countries since this disease is present in rodent populations and can be transmitted as an aerosol. Although a promising vaccine was in clinical development [115], [116], no vaccine is currently available for Lassa Fever.

Additionally, no known test has been developed for the Lassa virus. Viral RNA has not been quantified in saliva but has been detected in throat washings after ten days of those infected (Schmitz et al. 2002)[117]. However, the virus is detected in peak titers and is present in saliva in guinea pigs, an animal model used for the Lassa virus (Tang-Huau et 2019)[118]. In some cases, it can be potentially through human-to-human transmission (Lo Iacono et al. 2015)[119]. RT-LAMP has been used to detect the virus with a limit of 100 pg, which was 10-fold less than conventional RT-PCR and could effectively be used for detecting serum 10^6viral RNA molecules/ml found on day six after onset (Fukuma 2011)[120].

Nipah virus is also a zoonotic virus that spreads from bats to pigs and people. Then it can spread from person to person. This virus usually is found in the winter months in Malaysia, Singapore, Bangladesh, and India. Recently, an outbreak in India killed a 12-year-old boy, and health authorities have feared that Nipah could cause an epidemic as it can spread from human to human. After contracting the virus, symptoms can include fever, headaches, muscle pain, sore throat, and vomiting. After 24-48 hours without any intervention, this can progress to encephalitis, blindness, a coma, and potentially death, which can happen in 40-75% of human cases. The incubation time has usually been reported as 4 to 14 days. The virus has a high Ro of around 0.4 [121], which means it currently does not spread that fast, and with a high morbidity rate, the people that get it may succumb to the disease before applying. However, like any other virus, a mutation can increase the transmission between people.

Unfortunately, a vaccine or few interventions for treatment have been developed. A vaccine has been made to protect African Green Monkeys and is currently being tested)[122]. One drug, Ribavirin, can reduce mortality in acute cases [121]. Only passive immunization can stop someone from being infected currently.

The virus can be detected in several matrices, viz. cerebrospinal fluid, urine, blood, and throat washings [123]. Currently, they have used PCR-based screening for the virus, but since it requires a BSL-4 laboratory to do these tests, any exposure to the virus can lead to the death of the persons collecting the sample and doing the test; there needs to be some caution when handling. Currently, RT PCR is used to do this test in serum and can detect the virus down to 1 PFU. After the person has developed antibodies, ELISA can be used. Nipah virus is detected in throat washings in saliva from only 6 out of the 20 patients, and the presence of antibodies reduced the possibility of obtaining the virus [123]. A significant viral load exists in saliva since 50% of Nipah virus patients in Bangledesh developed the disease through person-to-person salivary transmission. However, to the author' knowledge, the RNA load does not seem to be quantified. Since the virus can spread through multiple transmission modes and kill the person before initiating an immune response, the best way to ensure reducing the spread of this virus would be to have community testing utilizing the LAMP technique modified to allow minimal training to run. Ma et al.[124] produced such a test for all known strains of Nipah virus, which can reach a limit of detection down to 100 pg of total Nipah Virus RNA. This turned out to be 10-fold higher than conventional PCR, but in this case, since the viral load in saliva; it is assumed that this should be sufficient to run in a community setting.

Zika Virus (ZIKV) belongs to the Flaviviridae family and Flavivirus genus [125]. It was identified in 1947, by the Rhesus monkey, in the Zika forest, Uganda, and the first description of infection in human beings was in 1954 in Nigeria [126][127]. Various casual diseases occurred for more than 50 years until ZIKV emerged in the Pacific and the Americas [125]. In 2007, a large outbreak was recorded on Yap Island (Micronesia) [128]. In 2015 the first case of Zika was reported in Brazil, one year after it had already spread to more than 20 countries in the Americas and the Caribbean [129]. The main route of the virus transmission occurs by the bite of the female mosquito of the genus Aedes, vertical transmission from the infected mother to the fetus, and by sexual contact with an infected person.[127] [130]. The person infected can be asymptomatic or present symptoms such as fever, rash, and arthralgia, which are non-specific and can be confused with other illnesses transmitted by the same vector, such as dengue and chikungunya [131], [132]. Diagnostics methods are based on enzyme immunoassays (ELISA), plate neutralization, and rapid tests. As for COVID-19, the current gold standard for emergent diseases is RT-qPCR to detect the virus's genetic material [133](Waggoner et al., 2016). Although it is a method that produces specific results, it is necessary to extract the viral RNA, and it requires expensive equipment and qualified professionals to perform and read the results. Serological methods can produce false positives due to cross-reaction with other viruses such as DENV[133], [134] Therefore, there is a need to develop fast and reliable tests, especially in areas with few resources, and RT-LAMP is a promising method for expanding diagnostic platforms for ZIKV [26], [109], [134]–[136][15]. Sabalza et al. used saliva samples to detect ZIKV by LAMP[136], associated with dot-blot and microfluidic device, being able to analyze up to 24 samples simultaneously, without the need of RNA extraction, demonstrating a limit of detection of $2.2x10^2$ (RNA copies/mL) of the technique.

Dengue virus is an arbovirus of the Flaviviridae family and the Flavivirus genus, there are four viral serotypes (DENV1, 2, 3, and 4), and the bite makes its

transmission in the female mosquito of the Aedes genus [137]. It is estimated that about 3.6 billion people are at risk of infection because they live in vector-endemic areas, and about 390 million infections occur yearly [138]. Due to the increasing world population density, urbanization, and changes in climatic conditions, it is estimated that in 30 years, there will be a much higher number of dengue cases [139]. Some symptoms of DENV infection are high fever, pain behind the eyes, headache, muscle, and joint pain, vomiting, and rash; findings on blood counts usually include thrombocytopenia and leukopenia [140]., 2012]. DENV diagnostic methods involve the detection of IgM, IgG, and IgA antibodies, detecting NS1 protein and amplifying genetic material by RT-PCR [141][World Health Organization, 2009]. Most current diagnoses are not feasible in resource-limited settings, as dengue is a common disease in regions with few financial resources [142]. Detection of the DENV virus in saliva has already been possible using RT-PCR [143] in IgG detection kits by ELISA [144]. The study by Humaidi et al. [145] demonstrated that it is possible to detect DENV RNA in saliva within the first three days of infection, with a higher specificity of 87.1% using semi-nested PCR. Most of the RT-LAMP detections in the literature used blood material as a sample, such as studies targeting the C-pRM gene [146] and the 3'-NCR gene detected through turbidity [147]and integrated into portable platforms of sequencing [148]. Given the similarity of symptoms of dengue, zika, and chikungunya [149] proposed a platform based on the isothermal amplification of RT-LAMP complexed with amplification reporting quenchers (QUARS) for the simultaneous detection of the three diseases and visualization through a smartphone.

Table 4 – Tropical Diseases

Disease	Biomarker	Biomarker Concentration in Saliva	LOD	References
Malaria	DNA	1.123 parasites/µL (95% CI, 0.55 - 2.294 parasites/µL)	1-2 parasites/µl	[6], [150]
Zika	DNA	7.44 x 10^4 ZIKV RNA.	2x10^3 copies/reaction	[125], [130], [136]
Dengue	RNA	Average of 2 × $10E^{-1}$ PFU/ml, 2 days after onset of symptoms	1.22 PFU	[109], [143]
Monkey Pox	DNA	Detected but not quantified	$10^{2.0-3.0}$ copies/reaction	[151], [152]
Lassa Fever	RNA	Detected after 8 days in saliva but not quantified.	20-38 copies	[117], [120]
Nipah	RNA	Detected in throat washing but not quantified	100 pg of total NiV pseudovirus RNA	[153], [154]
Marburg	RNA	Detected in throat washing but not quantified	10^2 copies per tube	[155], [156]
Ebola	RNA	on days 1–8, lowest CT value = 21, maximum log 105.5–106.3 copies/ml	243-290 copies	[157], [158]
Leishmania	DNA	Detected but not quantified in saliva	10^2 parasites/ml	[159], [160]
West Nile Virus	RNA	Detected in 2 of 34 but viral loads were too low to determine.	10^4 PFU	[161], [162]

3. Conclusions

Infectious diseases continue to pose a massive threat to a large proportion of the world's population, affecting people in all age groups. This is evident in the most recent pandemic caused by SARS-CoV-2, which challenged the global economic- and health system in both developed and developing countries. The emergence and re-emergence of new pathogens will potentially lead to other epidemics or pandemics in the future, emphasizing the importance of increased attention toward the field of novel diagnostics.

It is essential to establish a diagnostic tool that can be employed in a broad range of environmental settings to monitor and control disease spread worldwide,s, like the resource-limited settings evident in developing countries. Within this field, the LAMP detection tool has entered an exciting phase with increased interest and multiple studies highlighting its potential. The collective data reviewed through this paper have demonstrated the LAMP assay's ability to detect most of the presented pathogenic biomarkers with such sensitivity and specificity as the state-of-the-art methods. With this comparable or even higher detection performance outlined in the paper, the LAMP assay has displayed its potential as a detection tool for multiple infectious diseases.

A diagnostic method that can precisely determine pathogens work better if it is convenient for the persons getting screened and if there is an infrastructure for doing the tests. Using saliva as a matrix both increases the convenience of the tests and reduces the infrastructure needed. Saliva serves as a matrix with many advantages compared with the current state-of-the-art matrices due to its non-invasive manner and convenience. Recent studies have not adopted the idea of implementing saliva as the main sample matrix in LAMP, so pathogenic detection limits with other detection tools were reviewed as a comparison. With the systematic review of the literature through this paper, we demonstrate that there is still a gap or minimal existing knowledge of whether all the pathogens presented in this paper can be detected in saliva. The current studies investigating saliva as a matrix demonstrated that the detection level was comparable and, in some cases, even higher than the other matrices. These results would strongly indicate that the LAMP assay would apply to saliva samples of some of the pathogens. Further investigation of the detection levels of the pathogenic biomarkers in saliva could provide valid clinical data on disease presence in the matrix. It would be a viable option for low-resource areas to detect deadly pathogens.

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