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Lassa Virus Diagnostic Platforms: Limitations and Prospects

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Background: *Lassa virus* (LASV) is the cause of lassa fever (LF) belonging to *the Arenaviridae* family. Clinical diagnosis is often difficult because of symptoms commonality with other infectious diseases. Early and rapid diagnosis is critical for therapy initiation and LF transmission prevention and control.
Aims: This review aims to highlight current diagnostic platforms and prospects of new emerging sensitive platforms.
Methodology: Available published articles on LASV diagnostics with a focus on current methods: virus culture, enzyme-linked immunosorbent assay (ELISA), reverse transcriptase-polymerase chain reaction (RT – PCR) and rapid diagnostic tests (RDT) were reviewed based on their performances and limitations. Prospects of new diagnostic platforms: mobile health, microfluidic,

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clustered regularly interspaced short palindromic repeats (CRISPR)-associated (Cas), Loop-mediated isothermal amplification (LAMP) for LASV diagnosis were also reviewed.
Results: Low sensitivity of the ELISA platform during the window period of LASV infection was observed. Moreover, RT – PCR findings indicated limitation of expertise necessity, cost of thermal cycler, and dedicated facility. Molecular-based point-of-care (POC) diagnostic development should be prioritized to increase speed and sensitivity.
Conclusion: The integration of POC device into molecular isothermal method against LASV scourge will be a success story in curving intermittent outbreaks in endemic areas and prompt clinical management.

Keywords: Lassa virus; diagnostics; ELISA; PCR; LAMP; CRISPR-Cas.

1. INTRODUCTION

Lassa virus (LASV) is a single-stranded RNA (ssRNA) virus, and a novel member of *Arenaviridae* family that causes zoonotic and hemorrhagic lassa fever (LF) disease, occurring as both sporadic outbreak and endemic in West African countries including Sierra Leone, Guinea, Liberia, Ivory Coast, Mali and Nigeria (Fig. 1) [1]. The LASV has bi-segmented genome: made up of ~3.5 kb small RNA (sRNA) that codes for glycoprotein precursor (GPC) and nucleoprotein (NP) while the ~7.2 kb large RNA that codes for RNA-dependent RNA polymerase (LP) and the matrix protein (Z) [3]. The LASV is an important public health concern particularly in endemic areas with major symptoms of fever, muscle aches, sore throat, nausea, vomiting, chest, and abdominal pain [3,4]. The LASV was first discovered in Lassa town in 1969, named after the village, in Borno State, North-Eastern Nigeria. It is observed Following an outbreak in a missionary hospital, two nurses infected and died while the third case had a severe illness. Later, 14 health care workers were infected with a case fatality rate of 30 percent [5,6]. The virus was then identified and isolated in 1972, named *the Lassa virus* (Lassa mammarenavirus). Since then, several outbreaks have been reported in many African countries where the disease is considered endemic [7]. Nosocomial outbreaks indicated a case fatality burden of ~50 percent and the case fatality rate in endemic settings was reported to be 1 percent but claims more lives than Ebola because of annual higher incidence (300,000 to 500,000 cases) [7,8]. The pathogenesis of LASV is still poorly understood however, it causes generally a systemic disease that progresses to hemorrhage and organ failure [9]. Often, during the autopsy, the virus is being observed in many tissues, but the liver is the major target organ in humans. Mostly the infected person dies without any sign of hemorrhage because pathological and histopathological lesions do not seem to indicate any organ failure or death [9].

The early definitive diagnosis of LASV is paramount for the initiation of proper therapy, control measures, and clinical judgment. Multiple approaches to LASV detection include enzyme-linked immunosorbent assay (ELISA), reverse transcriptase-polymerase chain reaction (RT – PCR), and rapid diagnostic tests (RDT). However, these methods suffer limitations of cost, expertise, high turn-around-time (TAT), lack of results validation, and specificity compromise during the window period, in the case of ELISA [10]. Currently, authors are not aware of any LASV diagnostic validation studies showing assay performance using pan-LASV strains that cover diverse variations of Lassa genomes [11]. Good laboratory findings are useful and complement accurate epidemiological data gathering, community health safety, and awareness. The LASV clinical diagnosis is often difficult to establish due to the commonality of symptoms it shares with tropical diseases including malaria. Thus, laboratory results are necessary to establish causality [12]. Most of the LASV reports from the affected countries focused mainly on the management of outbreaks cases, neglecting the roles of diagnosis. This review aimed to highlight on advantages and limitations of current diagnostics methods as well as to elucidate the prospects of new emerging diagnostics.

1.1 Virus Lineages

Recent phylogenetic studies on LASV sRNA sequences have revealed the delineation of LASV into six major lineages differing based on geographical locations. For instance, three lineages (I, II, and III), are domiciled in Nigeria and lineage (IV) is found in Ivory Coast, Sierra Leone, Liberia, and Guinea with three distinct

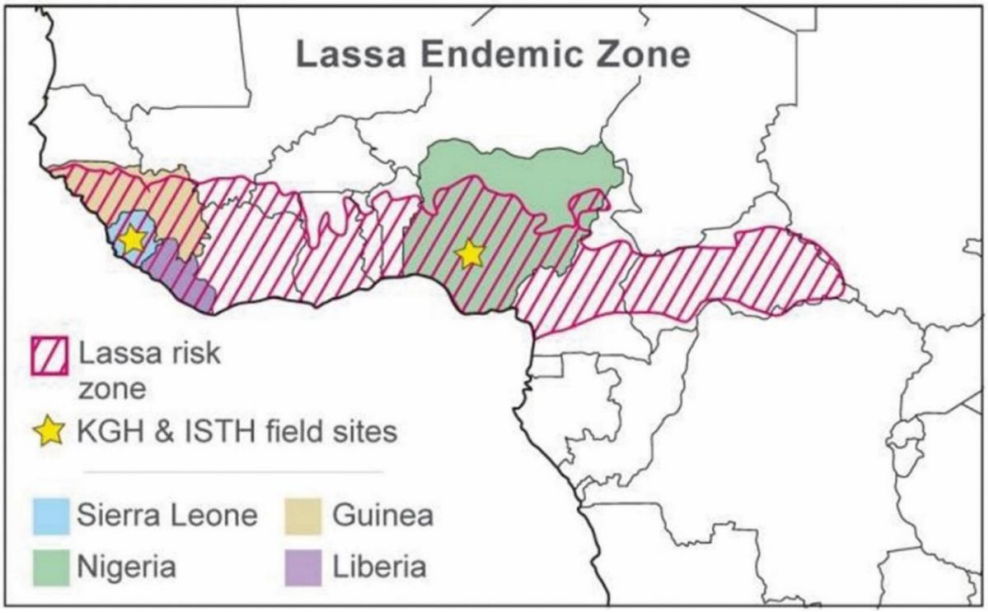

Fig. 1. Lassa fever endemic zones in West Africa [13] Legend: KGH: Kenema Government Hospital (Sierra Leone); ISTH: Irrua Specialist Teaching Hospital (Nigeria)

clades [1]. The fifth strain code-named "strain AV" was isolated from Mali/Cote D'Ivoire and Ghana [2,9] while Togo is linked to lineage VI [2]. The lineage I is the preliminary version of LASV isolated from North Eastern Nigeria, while Southern Central and Northern Central regions of Nigeria have II and III lineages [2,14]. However, serological surveys conducted in humans and rodents showed that LASV or its serologically related strains do exist in Cote d'Ivoire, Mali, Democratic Republic of Congo, Central Africa Republic, Ghana, and Burkina Faso, and believed to be imported from positive cases of endemic areas [8].

1.2 Virus Reservoir

Lassa virus has an African rat (*Mastomys natalensis*) as its natural host or primary reservoir, commonly distributed across Sub-Saharan Africa where the virus appears to be limited [15]. Recent studies reported that rodent species namely *Hylomyscus pamfi* and *Mastomys erythroleucus* harbor LASV [2]. *Mastomys* remains asymptomatic to infection despite the persistent presence of the virus, thus excretes the virus via urine and serves as a potential source of transmission to human through food contamination. The genetically diverse reservoirs continue to maintain distinct strains that allow cross-species transmission occurrence [16]. The LASV strain diversity is maintained through sustained rodent-to-rodent transmission chains [15]. The main reason behind the surprising surge in LF cases remains obscure, yet it might be associated with changes in the rat population or improved surveillance and sustained public awareness [16].

1.3 Human – To – Human Transmission

The majority of infections occur due to interactions between an animal reservoir or their excreta and human populations, or through nosocomial secondary transmission in humans [15,17]. Occasions of human-to-human transmission have been reported, especially in medical clinic settings, which is a focal point of concern. However, there are exemptions to the standard transmission design [16]. Of note, the direct contact with bodily secretions of infected persons is the major means of transmission [2]. The low transmission rate in humans suggests that rodents maintain largely the viral circulation in the environment [17]. A higher tendency of maternal mortality rate (29 – 80%) occurs in the third trimester of pregnancy. Vertical transmission of LASV during pregnancy results in fetal loss and neonatal death in 90 percent of cases. A study conducted by McElroy et al. [18] reported that the risk of sexual transmission of the virus after the detection of LASV in semen following symptomatic infection. In 2016, World Health Organization (WHO) [19] reported the first

case of LASV outside the African continent in an American evacuated from Togo to Germany on 25 February 2016 that died of multi-organ failure on 26 February 2016. The funeral home employee who handled the primary case's corpse became a secondary case as confirmed through RT – PCR testing.

1.4 Therapeutic Interventions

The recommended course of LASV management is shown to be generally supportive care with the management of specific symptoms commonly associated with LF. There is no approved antiviral therapy yet for LF; however, empirical therapy using ribavirin indicates good efficacy if administered at the early onset of symptoms. Delays in the initiation of ribavirin therapy cause an increase in case fatality rates [2]. Ribavirin has been shown to decrease the casualty in seriously sick patients with LF from 76 to 9 percent given that such patients get treatment within 7 days of the course of the disease [20]. Raabe et al. [21] viewed that initiation of treatment during the first 6 days of LF illness decreases mortality rates to 5 percent. Despite the history of teratogenicity, ribavirin should be given to infected pregnant women considering the high level of maternal and fetal mortality associated with LF during pregnancy [22]. Another novel antiviral drug with promising results on LF is favipiravir that has broad-spectrum activity against RNA viruses, which decreases the levels of LASV viremia and increases survival rate among the animal model tested [21].

1.5 Vaccine Development

The occasional or sometimes yearly resurgence of LF prompts the need for the development of rapid diagnostics, therapeutics, and vaccines [23]. Currently, there are no approved vaccines or therapeutics against the LASV, except for the use of empiric ribavirin [5]. However, efforts have been geared towards developing new ones that led to the formation of Coalition for Epidemic Preparedness Innovations (CEPI) committee in 2017, supported by donor agencies comprising of Wellcome Trust, national governments, and the Bill & Melinda Gates Foundation. The CEPI and its cohorts promised to push available vaccine candidates to phase III clinical trials in preparation for outbreak recurrence. Presently, there are few vaccine candidates which are on trial on non-human small primates [5].

2. METHODOLOGY

2.1 Current LASV Diagnostics

The effectiveness of current diagnostic platforms correlates the persistence of the virus and manifestation of symptoms inpatient that relatively affects the treatment protocols and subsequently may influence mortality rates [24]. Recent outbreaks in Nigeria indicate the need to promptly engage new methods of LASV diagnosis to tackle spread, lower morbidity, and mortality rates, due to limitations associated with current diagnostic platforms. Correct and speedy identification of the cause of acute febrile illness in endemic areas requires validated rapid and POC-based diagnostic techniques. The routine diagnostic techniques for LASV include:

2.2 Virus Culture Platform

The virus isolation from culture is considered as standard reference considering its isolation capability of diverse emerging LASV strains, despite the routine use of ELISA and highly sensitive and specific RT- PCR [11,25]. The virus can be isolated from different animal models such as guinea pigs, albino mice, African green monkeys, or cell culture such as Vero cells that show the cytopathic effect as a positive result [11,26]. The virus can be isolated from a vast array of patient's samples including blood, urine, pleural fluid, throat swab, and histopathological materials including liver, kidney, spleen, and heart, during autopsy [26]. The main advantage of this method is that it is independent of genetic variation among strains and is applicable for determining viremia quantification as well as allows further characterization and study on the virus [11]. The main limitation of this method is the requirement of high-level-security containment laboratories (BSL-IV) and takes about 4–7 days for effective viral growth in susceptible Vero E6 cells that delay initiations of ribavirin therapy to reduce the mortality tendency [20].

2.3 Enzyme-Linked Immunosorbent Assay (ELISA) Platform

Enzyme-linked immunosorbent assay is a promising diagnostic test for LASV, targeting specific antigens and/or IgM/IgG antibodies against the virus. The ELISA technique involvement of only viral antigen and the developed antibody justified its relative safety

when compared to RT-PCR that requires RNA extraction from the live virus. Although the LAMP substitutes indirect fluorescent antibody (IFA) technique due to its loss of sensitivity, it is commonly used in reference laboratories where BSL-IV facility is not readily available and it is cumbersome [27]. One of the striking limitations of the ELISA technique is the low sensitivity during the window period of LF infection because the body does not readily commence antibody production (IgM) in response to the presence of a virus. This may likely hinder early diagnosis and delay clinical judgment. The detection of both antigen and IgM indicated up to 88 percent sensitivity and 90 percent specificity of acute LF infection according to Dahmane et al. [28] Branco et al. [29] reported that IgM ELISA is no longer clinically significant because patients with other infections may indicate positive to IgM. The false-positive results of 8/35 (22.9%) cases detected in IgM antibody ELISA test were confirmed negative by more specific RT-PCR platform. This is corroborated by the findings of Ibekwe et al. [27] reported low sensitivity and specificity of ELISA technique to 57 percent and 77 percent respectively, as compared to RT-PCR that indicated 100 percent for both accuracy determinants. However, Boisen et al. [30] reported good performance of ELISA with 95 percent sensitivity and 97 percent specificity for LASV antigen detection from blood in comparison to immunoassays and qPCR as diagnostic standards. Thus, it is suggested that ELISA is never reliable for early LF diagnosis and delayed diagnosis may result in potential transmission to family members, health care workers and may be extended to the entire community [31]. Moreover, early diagnosis and initiation of therapy are extremely important for good clinical outcomes.

2.4 Reverse Transcription Polymerase Chain Reaction (RT-PCR) Platform

Lassa virus RNA is detected using various nucleic acid amplification methods, particularly conventional PCR in the presence of a reverse transcriptase enzyme that converts RNA to DNA [2]. The RT-PCR is a gold standard technique in molecular biology studies because it can amplify few copies to detectable copies of the target gene [15]. The RT-PCR method has the advantage of detecting the virus for a longer duration from body fluids [11]. The method is considered as a confirmatory test by some reference laboratories for instance, in Kenema Government Hospital in Liberia as reported by Hamblion et al. [32] The major gene targets in most of the studies include S segments gene that encodes for NP and GPC and L segment that encodes for RNA-dependent RNA polymerase (LP) and the matrix protein (Z). Ölschläger et al. [31] corroborated high analytical sensitivity of RT-PCR by detecting 15 LASV copies/reaction with a probability of 95 percent efficiency. However, its restriction to laboratory-based procedures, cost, machine dependence, and expertise, is its major limitations [33]. The RT-PCR technique is inadequate to detect different LASV lineages with high sensitivity due to strain variability emergence [14]. Lassa lineage diversity is of utmost concern due to the choice of PCR primers targeting the specific gene [34]. The sensitivity of RT-PCR relies on nucleic acid sequence homology along the gene [34]. Moreover, the dependence of specific primers in detecting specific target gene is also another limitation to mention, since it is likely to miss the gene if the copy number is low in the whole genome. Other limitations of conventional RT-PCR is that the method is prone to contamination due to the requirement of opening reaction tubes for detection in agarose gel [35]. The emergence of inter-strain lineages and clades with 32 percent and 25 percent variations for L segments and S segments, respectively showed the necessity of whole genome sequencing (WGS) application [36]. Nucleic acid sequence mutations necessitate constant validation to avoid missing target genes [2]. Another limitation worthy to note is the requirement for further sequencing analysis of PCR product to determine the presence of virus with certainty, despite agarose gel detection. The PCR requirement for instrumentation and pre-extraction step of RNA increases the cost and TAT with the consequences of extending the mortality rate of patients [37]. The quantitative reverse transcriptase PCR (RT-qPCR) for the detection of LASV is highly crucial for improving POC diagnosis and for surveillance and epidemiological control studies [38]. Both SYBR-Green and TaqMan probe methods are readily applicable for LASV detection. However, SYBR-Green has been regarded as technically simpler, less costly, and readily available. Although, less specific compared to the TaqMan probe or molecular beacon-based assays [39]. The less specificity of the SYBR-Green-based method is associated with fluorescent dyes that intercalate into any formed double-stranded DNA including primer-dimers and non-specific amplified products [39]. The main advantage of RT-qPCR is that it allows verification of all prior steps

of analysis including extraction, reverse transcription, and PCR amplification through melting and standard curve analyses [38]. The quantification capability of RT-qPCR also plays a significant role in strain identification. Accordingly, patients from Sierra Leone shows a higher viral load of LASV genome than those from Nigeria, perhaps due to increased codon optimization that leads to the increased viral output associated with their strain [40]. Moreover, the RT-qPCR has low TAT, less prone to contamination because it requires only computer-based analysis and no agarose gel electrophoresis analysis. However, despite these numerous mentioned advantages, the RT-qPCR has few limitations including the cost of a real-time thermal cycler, cost of reagents, and expertise dependence for the generation of standard and results interpretation [41].

2.5 Rapid Diagnostic Tests (RDTs) Platform

The RDT works in a similar principle with the ELISA method based on antigen/antibody capture, however, its reaction occurs on paper-based lateral flow strip that allows for POC testing during LF outbreaks [2]. Systematic and meta-analysis study conducted by Takah et al. [25] and reports of Boisen et al. [30] indicated one recombinant RDT test currently undergoing evaluation against Josiah strain (lineage IV) based on paired monoclonal antibodies that have good performance of 90 percent sensitivity and 100 percent specificity. This RDT showed high sensitivity close to that of RT – PCR with few false-positive results. Hartnett et al. [14] reported the development of POC rapid lateral flow strip that detects LASV antigen from 30 – 40 µL of blood, plasma, or serum sample using a finger prick. Another newly developed RDT targets NP of the virus directly from a whole blood finger prick [2]. Viral Hemorrhagic Fever Consortium (VHFC) is working currently on new clade-specific rapid diagnostics based on numerous sequenced data generated from LASV Nigerian strains to pass clinical validation and secure approval of regulatory bodies [14]. The RDT method has numerous advantages that provide easy-to-read qualitative test results, low TAT (10 – 30 min), easy transportation, and heat-stability. *Lassa virus* RDTs are less expensive in comparison to other diagnostic methods, thus facilitate use to screen and detect LF patients in epidemiological studies and outbreak containment [2,42]. Another advantage of this platform is the use low amount of blood which reduces the amount of blood collected from potentially anemic patients [14]. Moreover, simple blood collection from finger prick reduces the risk of transmission to health care providers [14]. The major concern about current RDT platforms is that they only target joshia strain, leaving other strain undetected. Another concern is the heat-stability that must be up to 40˚C or more to withstand the environmental temperature of endemic areas of Nigeria and other West African countries [25].

2.6 Prospects of New LASV Diagnostic Platforms

Diagnostics development researches are currently geared towards further improvements associated with sensitivity, specificity, miniaturization, cost reduction, and reducing reagents consumption. Below are some of the prospects in the possible advances in diagnostic researches against LASV.

2.7 CRISPR-Cas Platform

The CRISPR-Cas system was first discovered as an immune response developed by prokaryotes (bacteria and archaea) against bacteriophage attack and invading plasmids [43]. In this system, a Cas9 endonuclease splits double-stranded DNA in a specific site manner through the guidance of a short single-guide RNA (sgRNA). Cas9, thus, activates the double-strand break repair machinery called Non-Homologous End Joining repair (NHEJ), through cellular repair processes causing insertions or deletions (indels) or stimulating homologous recombination at the breaking point that eventually disrupt the targeted locus [44]. This process allows the editing of DNA sequence, allowing scientists to engineer nucleotide sequences through point mutations creation; regulatory regions substitutions; insertion of tags or reporter genes; exons deletion, or deletion of specific target genes [45]. It has major advantages of being programmable and applicable to different organisms, animal models, high-throughput technology, gene therapy, and many more [45]. Insertional mutagenesis in haploid cells has been utilized to find fundamental receptors for many viruses, including Ebola and LASV [46]. The CRISPR-Cas based assay showed POC characteristics including: (1) run a single reaction (2) available lyophilized reagents (3) < 2 h TAT (4) portable, simple and no requirement of electricity (5) direct detection from samples; and (6) a colorimetric detection [37].

2.8 Nanopore Sequencing Platform

Nanopore-based sequencing represents a unique approach from known polymerase-mediated sequencing, where the sequence of nucleic acids is deduced through interruption of ionic current passing across an α-hemolysin nanopore membrane of a single DNA molecule passing through it [47,48]. The technology has been commercialized in the form of a handheld MinION device developed by Oxford Nanopore Technologies and deployed widely for whole genome sequencing of bacteria, fungi, and viruses [48,49]. The amplitude recorded due to current passage interruption and span of this interference is dictated by the thickness and length of the DNA particles. Finally, the collapsed structure of a DNA or protein particle can be promptly deduced from the type of current barricade [50]. The technology provides simple, long reads products, portable, and no amplification-based sequencing tools, thus allowing field application [47,48,50]. The year 2018 in Nigeria, had been described as the year of LF upsurge outbreak with 1495 suspected cases and 376 confirmed cases across 18 states by 18th March according to study conducted by Kafetzopoulou et al. [36] One peculiar approach was the use of portable MinION for metagenomic sequencing of LASV during the outbreak for 120 samples. The main objective was to determine whether there was an emergence of a new strain that has a higher rate of transmission between humans. The findings indicated no new strain emergence and rodent was the main source of the outbreak.

2.9 Molecular – Based Point – of – Care (POC) Platform

Point-of-care (POC) is defined as paper-based tests performed at the side of patient care or in the field with four main distinguishing features from the traditional laboratory-based tests that include (1) non-requirement for laboratory infrastructure and sophisticated machines (2) easy to perform and interpret (3) low TAT and (4) less costly [51]. According to WHO, POC criteria were put together in the acronym "ASSURED"—affordable, sensitive, specific, user-friendly, rapid/robust, equipment-free, or minimal and deliverable to the concerned who are in dare need [52]. Molecular-based POC can be utilized in many health facilities to diagnose LF in patients during an outbreak response, clinical management, regular surveillance, and clinical trials in endemic areas [42]. The usual platform has been the lateral flow immunoassay (LFIA) and recent emergent molecular diagnostics for infectious diseases have met requirements for speed, low cost, and easy to use POC applications with a limited infrastructure [24]. The use of multiplex molecular-based POC on a single detection cassette that can detect various LASV lineages will be a potential solution to virus variability and will also help in epidemiological studies.

3. REVERSE TRANSCRIPTION ISO-THERMAL MOLECULAR PLATFORM INTEGRATED WITH LATERAL FLOW ASSAY

The relevance of PCR molecular-based diagnostics have been associated with cost, high TAT, and expertise dependence [53]. However, recent isothermal developed techniques such as LAMP [54], cross-priming amplification assay (CPA), helicase dependent amplification (HDA), strand displacement amplification (SDA), rolling circle amplification (RCA), self-sustained sequence replication (3SR) and nucleic acid sequence-based amplification (NASBA) have been developed [55]. The LAMP has been used for the detection of many infectious diseases and can be detected by the naked eye based on color change [56]. It requires no sophisticated equipment or dedicated facility to operate [57]. Such an approach will be critical for LASV detection particularly in remote areas away from reference centers to speed up patient care and clinical decisions. Fukuma et al. [58] reported good performance of RT-LAMP for LASV detection with the sensitivity of 100 copies of *in vitro* artificial transcribed LASV RNA in 25 minutes Mazzola and Kelly-Cirino [2] also reported the application of LAMP for detection of LASV due to its simplicity. The integration of LFA into molecular RT-LAMP avoids the use of an electronic reading system by allowing visual detection of target and increase the speed of detection. For example, two tags (e.g. biotin and digoxigenin) can be tagged to the specific primers before RT-LAMP amplification. The presence of amplified DNA can be detected by running the product on a lateral flow strip, containing the anti-digoxigenin on the test line that captures digoxigenin linked to amplified product and streptavidin on the control line to capture unconjugated biotin. Both RT-LAMP amplification and LFA detection can occur in < 2 h (Fig. 2).

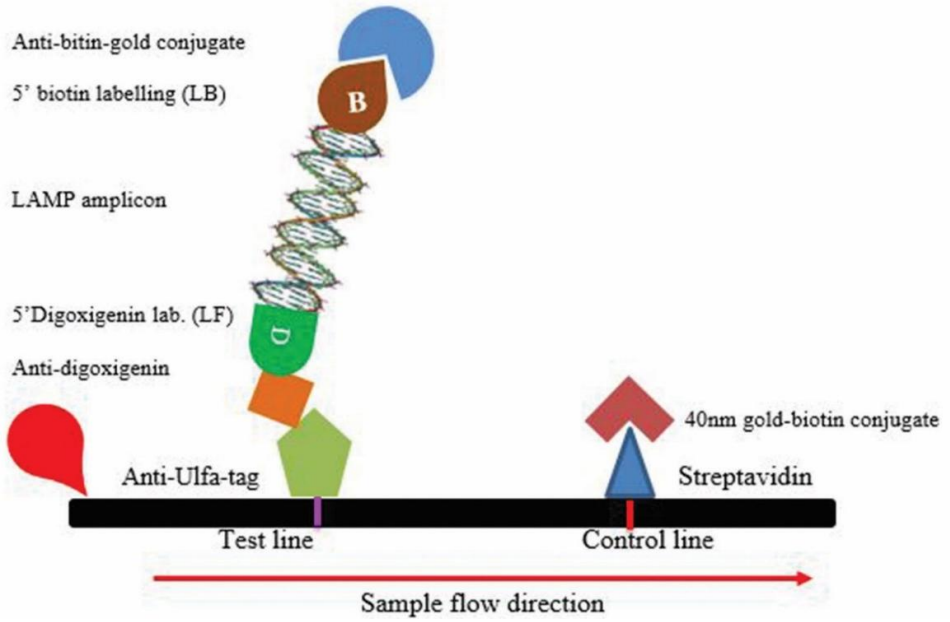

Fig. 2. Sketch lateral flow assay (LFA) strip for LAMP amplicon detection

3.1 Mobile Health Platform

Mobile health (m-health) has been defined according to the Global Observatory for electronic-health, a subsidiary of the World Health Organization (WHO), as "medical and public health practice supported by mobile devices such as mobile phones, patient monitoring devices, personal digital assistants and other wireless devices" (WHO, 2011) for data gathering. It is increasingly being used in public health for the prevention and control of diseases. Rapidity in transferring such data from these devices into an e-database or data management system will promote timely response to public health disease emergencies [59]. Lozano-Fuentes et al. [59] developed the approach for surveillance of immatures *Dengue virus* mosquito vectors in Mérida, México. The method allowed for field application in the endemic areas and this translated to the accuracy of detection as well as speed on the generation of transmission data for an adequate response to a dengue disease outbreak. Laktabai et al. [60] reported the application of m-health in assessing the diagnostic accuracy of rapid test kits for malaria diagnosis. The result indicated 91.6 percent concordance between the test kit and the m-health application. Accordingly, the m-health approach will be quite applicable to LF control, by tracking the rat vectors' hideouts and their excrements detection before the possible transmission and emergence of unwanted outbreaks. Moreover, innovation will be a cost-effective, user, and environmentally friendly.

3.2 Microfluidic Platform

Microfluidic technology is a new approach of POC that can overcome the limitation of conventional POC by providing improvements of fewer reagents consumption, increased portability, simplicity, and reduced reaction time [61]. It involves the preloading of the master mix containing all the reaction components in microliter volumes into a sample loading well where it moves into the reaction chamber and the amplicon detected at another segment of the setup. Many studies reported different setup, for example, Chen et al.[61] used in-gel microfluidic LAMP for multiplex detection of foodborne pathogens. Chung et al. [62] designed a microfluidic chip module for detecting murine norovirus in oysters using charge switchable micro-bead beating. Park et al. [16] reported the use of a rotary microfluidic system that involves multiplex sample-to-result application (DNA extraction to LFA detection) of foodborne bacterial isolates with a detection limit of 50 CFU in 80 minutes. The use of integrated microfluidic-POC set up if adopted for LASV detection will be an important platform due to simplicity and low cost.

4. GENERAL LIMITATIONS OF LASV DIAGNOSIS

4.1 Variability of LASV Strains

The high degree of genetic variations of LASV strains hinders specificity in molecular-based diagnostics. The genetic variability of LASV affects primer designing for RT-PCR assays that will reliably detect all emerging strains [34]. These may cause false-negative results if primers or probes are poorly designed with non-alignment to target sequence and consequently [34]. Ölschläger et al. [34] indicated the detection of variability in the genetic sequence of Liberian and Nigerian strains using a wobble base introduced at the – 6^{th} position of the primer (LVS-339-rev). Though successfully used in their study, it may likely not work in another variable strain.

4.2 Non-Availability of Containment Laboratories

The safety concern about the handling of LASV suspected specimens is paramount because of potential human-to-human transmission. Laboratory staff handling such potential infectious samples should be aware of the risk. Strict adherence to guidelines stipulated by the World Health Organization (WHO) for the collection, storage, and handling of Ebola specimens is also recommended for LASV since they share common infectivity status [63]. The BSL-IV laboratory is recommended for handling such infectious pathogens, which not readily available in most centers in affected countries including Nigeria. Moreover, BSL-IV laboratories are expensive to run and require expertise and security measures [24]. Moreover, electrical power instability complicates the application of BSL-IV in the region [30].

4.3 Cost of Developing Diagnostics

The alarming cost of developing new diagnostics with reduced TAT has also been part of the problems of LASV control measures. This is attributed to the requirement of thorough validation, meeting the ethical guidelines, and must be certified by standard regulatory bodies. Thus, it limits the timely availability of these diagnostics for routine clinical utilization in the health centers. The non-availability of highly sensitive and rapid test methods implies the easy spread of LASV in communities without being noticed, suggesting adverse consequences of raising its fatality rates [24,64].

5. CONCLUSION

Lassa fever poses a great threat to the large population of West Africa, particularly Nigeria that has the highest population in the region and where the virus emanates and circulates in three distinct lineages (I, II, and III). Difficulty in establishing prompt clinical diagnosis due to the commonality of symptoms with other commonly found infections in the endemic areas delays treatment initiation with grave consequences. Effective diagnostics are critical for prompt detection and containment of LF outbreaks. Enhanced simple, less costly, and low TAT platforms are necessary for clinical management, outbreak response, and therapeutic or vaccine clinical trials. Lassa fever has diagnostic assays for antigenemia, IgM, IgG, RT – PCR, and RDT detection. The goal of robust LF detection and control lies in field implementable molecular-based POC diagnostics for prompt therapeutic initiation and management. The POC holds promising results in reducing cost, improving simplicity, rapidity, and miniaturization. Moreover, the adoption of rapid isothermal platforms with POC integration would greatly simplify detection and prevents unnecessary exposure of health care personnel.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ehichioya DU, Hass M, Becker-ziaja B, Ehimuan J, Asogun DA, Fichet-calvet E, et al. Current Molecular Epidemiology of Lassa Virus in Nigeria §. 2011;49(3): 1157–61.
2. Mazzola LT, Kelly-Cirino C. Diagnostics for Lassa fever virus: A genetically diverse pathogen found in low-resource settings. BMJ Global Health. 2019;4(Suppl 2): e001116.

3. Freije CA, Myhrvold C, Boehm CK, Yozwiak NL, Zhang F, Sabeti PC, et al. Programmable inhibition and detection of RNA viruses using cas13 resource programmable inhibition and detection of RNA viruses using Cas13. Molecular Cell [Internet]. 2019;76(5):826-837.e11. Available:https://doi.org/10.1016/j.molcel.2019.09.013
4. Olugasa B, Ojo J, Odigie E, Lawani M. Development of a time-trend model for analyzing and predicting case-pattern of lassa fever epidemics in Liberia, 2013-2017. Annals of African Medicine [Internet]. 2015;14(2):89. Available:http://www.annalsafrmed.org/text.asp?2015/14/2/89/149892
5. Warner BM, Safronetz D, Stein DR. Current research for a vaccine against Lassa hemorrhagic fever virus. Drug Design, Development, and Therapy [Internet]. 2018;12:2519–27. Available:https://www.dovepress.com/current-research-for-a-vaccine-against-lassa-hemorrhagic-fever-virus-peer-reviewed-article-DDDT
6. Ilesanmi OS, Omotoso B, Alele FO, Adewuyi P. Awareness of Lassa fever in a rural community in southwest Nigeria. Scholars Journal of Applied Medical Sciences. 2015;3(1):1137–42.
7. Kerber R, Reindl S, Romanowski V, Gómez RM, Ogbaini-Emovon E, Günther S, et al. Research efforts to control highly pathogenic arenaviruses: A summary of the progress and gaps. Journal of Clinical Virology [Internet]. 2015;64:120–7. Available:http://dx.doi.org/10.1016/j.jcv.2014.12.004
8. Safronetz D, Lopez JE, Sogoba N, Traore' SF, Raffel SJ, Fischer ER, et al. Detection of Lassa Virus, Mali. Emerging Infectious Diseases [Internet]. 2010;16(7):1123–6. Available:http://wwwnc.cdc.gov/eid/article/16/7/10-0146_article.htm
9. Oloniniyi OK, Unigwe US, Okada S, Kimura M, Koyano S, Miyazaki Y, et al. Genetic characterization of Lassa virus strains isolated from 2012 to 2016 in southeastern Nigeria. PLoS Neglected Tropical Diseases. 2018;12(11):1–13.
10. Greenky D, Knust B, Diseases I, Control D, Dziuban EJ. What pediatricians should know about the lassa virus. 2018;30333.
11. Raabe V, Koehler J. Laboratory diagnosis of Lassa fever. Journal of Clinical Microbiology. 2017;55(6):1629–37.
12. Raabe V, Koehler J. Laboratory diagnosis of Lassa fever. Journal of Clinical Microbiology. 2017;55(6):1629–37.
13. Yozwiak NL, Happi CT, Grant DS, Schieffelin JS, Garry RF, Sabeti PC, et al. Roots, not parachutes: Research collaborations combat outbreaks. Cell. 2016;166(1):5–8. Available:http://dx.doi.org/10.1016/j.cell.2016.06.029
14. Hartnett JN, Boisen ML, Oottamasathien D, Jones AB, Millett MM, Nelson DS, et al. Current and emerging strategies for the diagnosis, prevention, and treatment of Lassa fever. Future Virology. 2015;10(5):559–84.
15. Andersen KG, Shapiro BJ, Matranga CB, Sealfon R, Lin AE, Moses LM, et al. Clinical Sequencing Uncovers Origins and Evolution of Lassa Virus. Cell. 2015;162(4):738–50.
16. Siddle KJ, Eromon P, Barnes KG, Mehta S, Oguzie JU, Odia I, et al. Genomic Analysis of Lassa Virus during an Increase in Cases in Nigeria in 2018. New England Journal of Medicine. 2018;NEJMoa1804498. Available:http://www.nejm.org/doi/10.1056/NEJMoa1804498
17. Mylne AQN, Pigott DM, Longbottom J, Shearer F, Duda KA, Messina JP, et al. Mapping the zoonotic niche of Lassa fever in Africa. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2015;109(8):483–92.
18. McElroy AK, Akondy RS, Harmon JR, Ellebedy AH, Cannon D, Klena JD, et al. A case of human Lassa virus infection with robust acute T-cell activation and long-term virus-specific T-cell Responses. Journal of Infectious Diseases. 2017;215(12):1862–72.
19. World Health Organization (WHO). The WHO calls for early diagnostic tests for Lassa; 2020. Available:https://www.who.int/emergencies/diseases/lassa-fever/early-diagnostic-lassa-fever/en (Accessed June 2020.)
20. Demby AH, Chamberlain J, Brown DWG, Clegg CS. Early diagnosis of Lassa fever by reverse transcription-PCR. Journal of Clinical Microbiology. 1994;32(12):2898–903.
21. Raabe VN, Kann G, Ribner BS, Andres AM, Varkey JB, Mehta AK, et al. Favipiravir and ribavirin treatment of

epidemiologically linked cases of lassa fever. Clinical Infectious Diseases. 2017; 65(5):855–9.
22. Grant DS, Khan H, Schieffelin J, Bausch DG. Chapter 4 – Lassa fever. In: Emerging Infectious Diseases. 2014;37-59.
23. Okoror LE, Okoror OI. Molecular evolutionary studies of Lassa virus nucleoprotein. 2011;2(1):1–5.
24. Wilkinson A. Emerging Disease or Emerging Diagnosis? Lassa Fever and Ebola in Sierra Leone. Anthropological Quarterly. 2017;90(2):369–97. Available:https://muse.jhu.edu/article/663619
25. Takah NF, Brangel P, Shrestha P, Peeling R. Sensitivity, and specificity of diagnostic tests for Lassa fever: A systematic review. BMC Infectious Diseases. 2019;19(1):1–11.
26. Nasir I, Sani F. Outbreaks, pathogen containment and laboratory investigations of Lassa fever in Nigeria: How prepared are we? International Journal of TROPICAL DISEASE & Health [Internet]. 2015;10(1):1–10. Available:http://sciencedomain.org/abstract/10027
27. Ibekwe T, Nwegbu M, Okokhere P, Adomeh D, Asogun D. The sensitivity and specificity of Lassa virus IgM by ELISA as a screening tool at the early phase of Lassa fever infection. Nigerian Medical Journal. 2012;53(4):196. Available:http://www.nigeriamedj.com/text.asp?2012/53/4/196/107552
28. Dahmane A, van Griensven J, Van Herp M, Van den Bergh R, Nzomukunda Y, Prior J, et al. Constraints in the diagnosis and treatment of Lassa Fever and the effect on mortality in hospitalized children and women with obstetric conditions in a rural district hospital in Sierra Leone. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2014; 108(3):126–32.
29. Branco LM, Boisen ML, Andersen KG, Grove JN, Moses LM, Muncy IJ, Henderson LA, Schieffellin JS, Robinson JE, Bangura JJ, Grant DS. Lassa hemorrhagic fever in a late-term pregnancy from northern Sierra Leone with a positive maternal outcome: Case report. Virology Journal. 2011;1;8(1):404. Available:http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed13&NEWS=N&AN=51576375
30. Boisen ML, Hartnett JN, Shaffer JG, Goba A, Momoh M, Sandi JD, et al. Field validation of recombinant antigen immunoassays for diagnosis of Lassa fever. Scientific Reports. 2018;8(1):1–14.
31. Raabe V, Koehler J. Laboratory Diagnosis of Lassa Fever. Kraft CS, editor. Journal of Clinical Microbiology. 2017;55(6):1629–37. Available:http://jcm.asm.org/lookup/doi/10.1128/JCM.00170-17
32. Hamblion EL, Raftery P, Wendland A, Dweh E, Williams GS, George RNC, et al. The challenges of detecting and responding to a Lassa fever outbreak in an Ebola-affected setting. International Journal of Infectious Diseases. 2018;66: 65–73. Available:https://www.sciencedirect.com/science/article/pii/S1201971217302898?dgcid=raven_sd_recommender_email
33. Njiru ZK. Loop-mediated isothermal amplification technology: Towards point of care diagnostics. PLoS Neglected Tropical Diseases. 2012;6(6):1–4.
34. Ölschläger S, Lelke M, Emmerich P, Panning M, Drosten C, Hass M, et al. Improved detection of Lassa virus by reverse transcription-PCR targeting the 5′ region of S RNA. Journal of Clinical Microbiology. 2010;48(6):2009–13.
35. Asogun DA, Adomeh DI, Ehimuan J, Odia I, Hass M, Gabriel M, et al. Molecular Diagnostics for Lassa Fever at Irrua Specialist Teaching Hospital, Nigeria: Lessons Learnt from Two Years of Laboratory Operation. PLoS Neglected Tropical Diseases. 2012;6(9).
36. Leone S. Metagenomic sequencing at the epicenter of the Nigeria 2018 Lassa fever outbreak. 2019;77:74–7.
37. Chiu C. Cutting-Edge Infectious Disease Diagnostics with CRISPR. Cell Host and Microbe. 2018;23(6):702–4. Available:https://doi.org/10.1016/j.chom.2018.05.016
38. Dedkov VG, Magassouba N 'Faly, Safonova MV., Naydenova EV., Ayginin AA, Soropogui B, et al. Development and evaluation of a one-step quantitative RT-PCR assay for detection of Lassa virus. Journal of Virological Methods [Internet]. 2019;271:113674. Available:https://doi.org/10.1016/j.jviromet.2019.113674
39. Liu Z, Wang F, Yuan L, Zhang X, Ying Q, Yu L, et al. Development of an SYBR-Green quantitative PCR assay for the

detection and genotyping of different hantaviruses. International Journal of Molecular Medicine. 2016;38(3):951–60.
40. Andersen KG, Shapiro BJ, Matranga CB, Sealfon R, Lin AE, Moses LM, et al. Clinical Sequencing Uncovers Origins and Evolution of Lassa Virus. Cell. 2015; 162(4):738–50.
41. Dorak T. Real-Time PCR.
42. Emperador DM, Yimer SA, Mazzola LT, Norheim G, Kelly-Cirino C. Diagnostic applications for Lassa fever in limited-resource settings. BMJ Global Health. 2019;4(Suppl 2):e001119.
43. Chen S, Yu X, Guo D. Strategy A. CRISPR-Cas targeting of host genes as an antiviral strategy. 2018;(Lv):1–28.
44. Enkler L, Richer D, Marchand AL, Ferrandon D, Jossinet F. Genome engineering in the yeast pathogen Candida glabrata using the CRISPR-Cas9 system. Scientific Reports. 2016;6(May):1–12. Available:http://dx.doi.org/10.1038/srep35766
45. Pawluk A. CRISPR: No Sign of Slowing Down. Cell. 2018;174(5):1039–41. Available:https://doi.org/10.1016/j.cell.2018.08.010
46. Puschnik AS, Majzoub K, Ooi YS, Carette JE. A CRISPR toolbox to study virus-host interactions. Nature Reviews Microbiology. 2017;15(6):351–64.
47. Bowden R, Davies RW, Heger A, Pagnamenta AT, de Cesare M, Oikkonen LE, et al. Sequencing of human genomes with nanopore technology. Nature Communications. 2019;10(1): 1–9. Available:http://dx.doi.org/10.1038/s41467-019-09637-5
48. Wang Y, Yang Q, Wang Z. The evolution of nanopore sequencing. Frontiers in Genetics. 2014;5:1–20.
49. Deamer DW. Characterization of individual polynucleotide molecules using a membrane channel. Chemtracts. 1997; 10(3):255–7.
50. Lim MC, Kim YR. Analytical applications of nanomaterials in monitoring biological and chemical contaminants in food. Journal of Microbiology and Biotechnology. 2016; 26(9):1505–16.
51. Drancourt M, Michel-Lepage A, Boyer S, Raoult D. The Point-of-Care Laboratory in Clinical Microbiology. Clin Microbiol Rev. 2016;29(3):429–47.
52. Wu Z, Fu Q, Yu S, Sheng L, Xu M, Yao C, et al. Pt@AuNPs integrated quantitative capillary-based biosensors for point-of-care testing application. Biosensors and Bioelectronics. 2016;85:657–63.
53. Kozel TR, Burnham-marusich AR. crossm Diseases: Past, Present, and Future. 2017; 55(8):2313–20.
54. Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N, et al. Loop-mediated isothermal amplification of DNA. Nucleic acids research. 2000;28(12): E63. Available:http://www.ncbi.nlm.nih.gov/pubmed/10871386%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC102748
55. Wang DG, Brewster JD, Paul M, Tomasula PM. Two methods for increased specificity and sensitivity in loop-mediated isothermal amplification. Molecules. 2015;20(4):6048–59.
56. Xu X, Wang X, Hu J, Gong Y, Wang L, Zhou W, et al. A smartphone-based on-site nucleic acid testing platform at point-of-care settings. 2019;914–21.
57. Notomi et al. Loop-mediated isothermal amplification (LAMP): Principle, features, and prospects. Journal of Microbiology. 2015;53.
58. Aiko Fukuma, Yohei Kurosaki, Yuko Morikawa, Allen Grolla, Heinz Feldmann and JY. NIH public access. 2012;55(1): 1–12.
59. Lozano-Fuentes S, Wedyan F, Hernandez-Garcia E, Sadhu D, Ghosh S, Bieman JM, et al. Cell phone-based system (Chaak) for surveillance of immatures of dengue virus mosquito vectors. Journal of Medical Entomology. 2013;50(4):879–89. Available:http://www.ncbi.nlm.nih.gov/pubmed/23926788%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3929104
60. Laktabai J, Platt A, Menya D, Turner EL, Aswa D, Kinoti S, et al. A mobile health technology platform for quality assurance and quality improvement of malaria diagnosis by community health workers. PLoS ONE. 2018;13(2):1–14.
61. Chen C, Liu P, Zhao X, Du W, Feng X, Liu BF. A self-contained microfluidic in-gel loop-mediated isothermal amplification for multiplexed pathogen detection. Sensors and Actuators, B: Chemical. 2017;239: 1–8. Available:http://dx.doi.org/10.1016/j.snb.2016.07.164

62. Chung SH, Baek C, Cong VT, Min J. The microfluidic chip module for the detection of murine norovirus in oysters using charge switchable micro-bead beating. Biosensors and Bioelectronics. 2015;67: 625–33.
63. Ölschläger S, Lelke M, Emmerich P, Panning M, Drosten C, Hass M, et al. Improved detection of Lassa virus by reverse transcription-PCR targeting the 5′ region of S RNA. Journal of Clinical Microbiology. 2010;48(6):2009–13.
64. Shaffer JG, Grant DS, Schieffelin JS, Boisen ML, Goba A, Hartnett JN, et al. Lassa Fever in Post-Conflict Sierra Leone. Bird B, editor. PLoS Neglected Tropical Diseases. 2014:20;8(3):e2748. Available:https://dx.plos.org/10.1371/journal.pntd.0002748

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