Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Virology

Lassa fever diagnostics: past, present, and future

Anise N Happi[1], Christian T Happi[2,3] and Randal J Schoepp[4]

Lassa fever is a unique viral hemorrhagic fever that is endemic in parts of West Africa, primarily Sierra Leone, Guinea, Liberia, and Nigeria. The disease is caused by the Lassa virus, an Old World arenavirus that has as primary reservoir host the multimammate rodent *Mastomys nataliensis*, which lives in association with humans. Recent estimates suggest LF causes two million cases and 5000–10 000 deaths annually, mainly in West Africa.

Clinical diagnosis and laboratory confirmation have always been major challenges for effective management and control of the disease in afflicted areas of West Africa. Recent advancements in molecular biology, recombinant DNA technology, and genomics sequencing has facilitated major advancement in development of better diagnostic and surveillance tools for Lassa fever virus. These include, the multiplex, magnetic bead-based immunodiagnostics for both Lassa virus antigens and antibodies; molecular probe-based quantitative real-time PCR for genomic signatures; rapid diagnostics tests that detects the most prevalent West African lineages; and the successful utilization of next-generation sequencing technology to diagnose and characterize Lassa virus in West Africa. These advances will continue to improve disease treatment, control, and prevention.

In this review we will discuss progression of Lassa virus diagnostics from the past and into the future.

Addresses
[1] Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria
[2] Department of Biological Sciences, College of Natural Sciences, Redeemer's University, Ede, Osun State, Nigeria
[3] African center of Excellence for Genomics of Infectious Diseases (ACEGID), Redeemer's University, Ede, Osun State, Nigeria
[4] U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland, USA

Corresponding author: Schoepp, Randal J (randal.j.schoepp.civ@mail.mil)

Current Opinion in Virology 2019, **37**:132–138

This review comes from a themed issue on **Lassa viruses**

Edited by **Connie Schmaljohn** and **David Safronetz**

For a complete overview see the Issue and the Editorial

Available online 11th September 2019

https://doi.org/10.1016/j.coviro.2019.08.002

Introduction

Lassa fever (LF) is a unique viral hemorrhagic fever regularly reoccurring in endemic parts of West Africa, primarily Sierra Leone, Guinea, Liberia, and Nigeria. The name derives from Lassa, a village in Nigeria where the first cases occurred in 1969 [1–3]. Lassa virus (LASV), is a member of the *Arenaviridae* which contains both Old World and New World species [4,5]. LASV is an Old World arenavirus, and its primary reservoir host is the multimammate rodent *Mastomys nataliensis*, which lives in association with humans [6]. Secondary rodent reservoirs include the African wood mouse *Hylomyscus pamfi* and the Guinea multimammate mouse *Mastomys erythroleucus* [7]. Human infections primarily occur through contact or ingestion of contaminated foodstuffs with infected rodent excreta, urine, or blood. Infections may also occur through inhalation of aerosols contaminated with dried excreta or urine of infected rodents. Human to human transmission can occur through contact with blood or body fluids during care for the sick or deceased.

Recent estimates suggest LF causes two million cases and 5000–10 000 deaths annually [8,9]. Humans in LF endemic areas have high prevalence of LASV-specific antibodies suggesting many infections are mild or asymptomatic [10]. Acute LF causes a range of symptoms that in the early stages can mimic other more common endemic diseases.

Lassa fever has an incubation period of 6–21 days and begins with generalized flu-like symptoms of fever, weakness, cough, sore throat, and joint, back and chest pain [1,10,11]. In severe cases, this may progress to abdominal pain, vomiting, diarrhea, pharyngitis and conjunctivitis. Late stage symptoms may include respiratory difficulty, facial edema and hearing loss. Generally, recovery begins 8–10 days post-onset. However, a small percentage of infections progress to acute hemorrhagic fever with multi-organ failure [12]. Case fatality rates may be 1–15%. Significant predictors of fatal outcome are hemorrhage, sore throat, and viremia [13]. Early detection and identification are critical because treatment with the anti-viral Ribavirin must be administered within six days of infection for greatest effectiveness [14].

Since the early stages of LF are similar to other common diseases, effective treatment requires early diagnosis and immediate use of Ribarvirin. In this chapter we will discuss progression of Lassa virus diagnostics from the past and into the future.

Genetics of Lassa virus

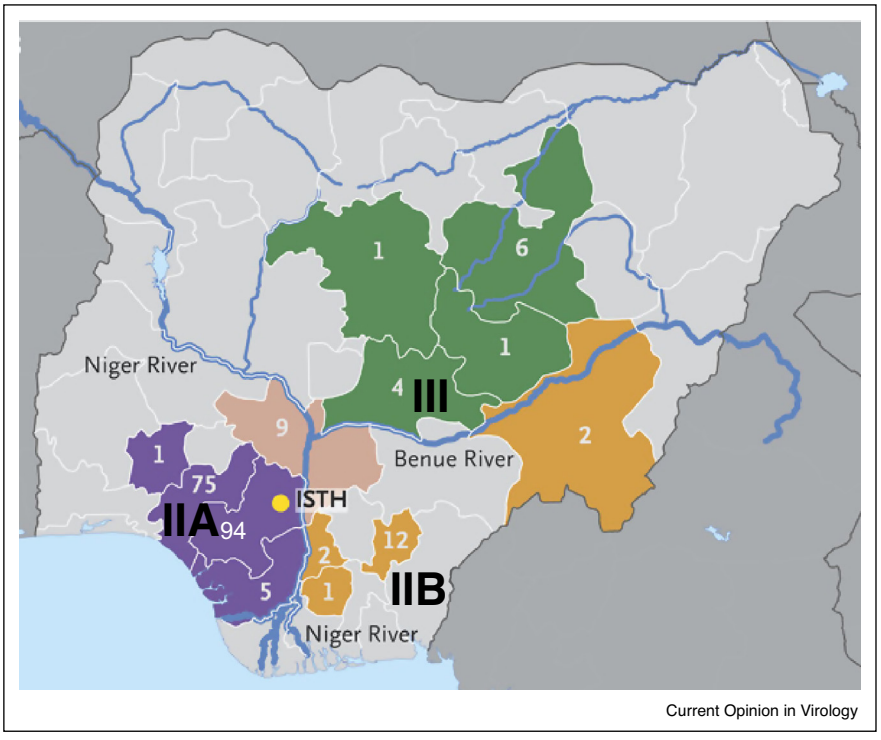
LASV is an enveloped negative-sense, single-stranded RNA virus. The genome comprises two ambisense segments (the large and small segments). The large segment encodes the RNA polymerase (L) and a zinc-binding protein (Z), which is the equivalent of the matrix protein in other RNA viruses [15]. The small segment encodes the nucleoprotein (NP) and the envelope glycoprotein complex (GPC). Coding regions on each segment are separated by an intergenic (IGR) non-coding region that forms a stable loop (hairpin) [16]. The IGR functions in structure-dependent transcription termination and in virus assembly and/or budding.

The NP is the major structural protein, and comprises nucleocapsid proteins that play essential roles in replication and transcription of viral RNA and in virion assembly. It is used to evade the RIG-I-like pathway of the innate immune response by binding to the kinase domain of IKK-ε [17]. The NP is the most sequenced part of the LASV genome and is useful in classification of various strains and lineages. The GPC gives rise to the envelope protein that mediates viral attachment and cell entry. It is proteolytically processed by host cell subtilase SKI-1/S1P (13) into a heterotrimer in the lipid bilayer of the mature virion. Each heterotrimer consists of a receptor-binding GP1 domain, a GP2 class I membrane fusion protein, and a myristoylated stable signal peptide (SSP), which is required for GPC processing and function [18]. The L RNA polymerase functions in transcription and replication while the Z protein forms the matrix layer of the virions.

Lineages

Lassa viruses can be classified into five lineages, I–V. The nucleotide and amino acid divergence among the viruses is up to 27 and 15%, respectively [19]. Lineages I–III are commonly found in Nigeria, though lineage I has not been reported in nature for over 40 years; lineage IV, represented by the Josiah strain, occurs across West Africa; and lineage V is found in Mali and Ivory Coast [20,21•]. Geography plays a role in delineating Lassa virus lineages in Nigeria [22••]. Lineages II and III are stably separated along the course of the two big rivers in Nigeria, the Niger and the Benue. Lineage II occurs south of the Benue and Niger Rivers and lineage III to the north. Lineage II has further evolved into lineage IIA mainly occurring south west of the Niger and lineage IIB confined to south east of the river (Figure 1). The bottleneck that has confined lineages I–III in Nigeria allowing lineage IV to travel across West Africa is yet to be ascertained.

Figure 1



Lassa fever lineage distribution across Nigeria. Next generation sequencing of Lassa virus revealed that the virus lineages in Nigeria are separated geographically by major natural boundaries, which are the rivers Niger and Benue. Lineage III is found in the north (green colour) and separated from lineage IIB (Orange colour) in the South East and South-South by river Benue, and lineage IIA (Purple colour) in the South West by river Niger. The numbers in the graph represent number of successful complete Lassa virus sequences performed at each of the localities.

Laboratory diagnosis

The clinical symptoms of LF are unspecific, giving a wide range of differentials particularly in the early phase of the disease. This renders the laboratory diagnosis necessary and paramount for rapid and accurate intervention.

Virus isolation is the gold standard for LASV diagnostics, but is impractical in endemic areas due to the requirement for BSL-4 biocontainment. Detection of viral proteins or LASV-specific IgM or IgG antibodies by enzyme-linked immunosorbent assay (ELISA) or nucleic acids by reverse transcriptase-PCR (RT-PCR) are the most common methods used today. In the past, immunofluorescence assay (IFA) was used to detect LASV antibodies, but the requirements for BSL-4 biocontainment, highly trained technicians, and inferior sensitivity limited use. However, it remains in use in clinical and laboratory settings [23].

PCR-based assays

PCR-based molecular assays such as RT-PCR are an important part of an orthogonal diagnostic system providing the greatest confidence in results [24,25]. These sensitive and specific assays are the method of choice for detection of LASV genomic material [25–34]; however, PCR is not always available in endemic areas [35]. In addition, the high specificity of PCR-based assays can be problematic due to the considerable genetic variation in LASV strains over a wide geographic area, which increases the potential for primer and/or probe failures. The majority of assays target the small RNA that encodes the glycoprotein complex (GPC) precursor and the nucleoprotein (NP) [26–29,31,32,34]. With the availability of more sequence information, assays that target the large RNA segment coding for the RNA polymerase (L) and the matrix protein (Z) have been developed [30].

PCR-based assays are technologically complex compared to immunological assays. Standard RT-PCR assays using only amplifying primers are commonly used due to their lower complexity [26,27,30,33]. To add more specificity, probe-based real-time RT-PCR assays employing amplifying primers and a specific-labeled probe that hybridizes the amplicon, increases the assay specificity over primers alone [31,32,34]. While more technologically complex, a real-time PCR instrument controls temperatures, collects data, analyzes results, and therefore has more capacity and faster through-put than gel-based standard PCR. Unfortunately, the exquisite specificity of the primer and probe-based assays combined with LASV genetic diversity unavoidably increases the chance of genetic mismatches and potential false negatives [36].

Antibody-based assays

ELISA is a robust and simple method that can be performed in relatively austere laboratories in LF endemic countries. Detection of antigen or LASV-specific IgM antibodies has clear clinical significance for patient diagnosis. Detection of LASV-specific IgG has limited utility in patient diagnosis because IgG is produced later in the infection; however, this assay is often used for disease surveillance.

Detection of antigen generally employs a capture or sandwich ELISA. A 'capture' antibody conjugated to a solid support binds LASV in a sample. After washing away non-binding components, the captured antigen is 'sandwiched' by a primary antibody. Addition of a secondary (antispecies-specific) antibody conjugated to a reporter molecule produces a detectable signal. IgM, the first and thus most clinically relevant antibody produced after infection, can also be detected using sandwich ELISA. In this case, the LASV antigen bound by the capture antibody is exposed to a serum sample containing LASV-specific IgM. The IgM binds the captured LASV and the complex is detected by a species-specific anti-IgM antibody conjugated to a reporter molecule.

These assays traditionally are performed with whole inactivated virus or virus-infected cell lysate as the positive control in the antigen capture ELISA or the IgM target in the capture IgM ELISA. The need for LASV inactivated material makes these assays difficult to sustain due to the requirement for BSL-4 biocontainment to produce, inactivate, and safety test antigen. Today, recombinant antigens can be used in place of virus preparations. These recombinants can be easily expressed in large quantities and don't require biocontainment; however, comparison of recombinant-based assays to live and/or inactivated virus-based assays is always prudent.

Boisen *et al.* prepared a LASV antigen capture ELISA (ReLASV) using an affinity purified rabbit NP polyclonal antibody produced by immunization with a recombinant NP. In a comparison to RT-PCR they found a sensitivity of 94% and specificity of 84% against the benchmark PCR-based assay [37]. When combined with a lateral flow immunoassay (LFI) utilizing paired monoclonal antibodies also raised against recombinant NP, the sensitivity and specificity of both assays were 95% and 97%, respectively, compared to the benchmark RT-PCR assay.

Gabriel *et al.* developed LASV-specific indirect antibody-capture ELISAs using recombinant NP as the target to detect IgM and IgG and compared it to IFA [38••]. Clinical sample testing by ELISA was comparable to IFA. Stand-alone IgM ELISA in absence of IgG had little diagnostic value and IgM and IgG testing results combined were of low sensitivity (26%).

The host response to LF infection remains incompletely understood. In most virus infections, IgM is initially produced, wanes, and is followed by an increase of IgG that persists long after the infection resolves. Some

observations suggest that LASV infections can result in production of IgM and IgG nearly simultaneously [38••,39]. Wulff and Johnson found LASV IgG antibodies detected by IFA occurred at the same time or only slightly later than IgM. Similarly, Gabriel *et al.* in a much larger study, found some patients produced IgG just before IgM. Contrary to dogma, LASV IgM may persist for years [40]. In non-endemic LF regions of Sierra Leone, sera of normal healthy donors with no recent febrile illnesses, had high levels of LASV-specific IgM, suggesting that IgM may not correlate with acute LASV infection. Therefore, for early detection of LF, if virus isolation is not feasible, the preferred methods are RT-PCR or sensitive antigen detection [23,25–27,29,30,34,38••]

Lateral flow immunoassays

In resource limited regions, methods such as virus isolation, RT-PCR, and even ELISA can be challenging due to their technological and power requirements. In a perfect world, a point-of-care (POC) device would be easily transported without a cold chain, performed in minutes, and interpreted by a user with limited training. Lateral flow immunoassays (LFI) meet these criteria; however they often exhibit low sensitivity and can be prone to false positives. In an outbreak setting, LFIs can make a significant difference in the course of the epidemic due to rapid control and prevention [41•]. With the addition of a rapid diagnostic test that could be performed in the field with blood from a finger prick, by technicians with little training, and no cold chain requirement, it is estimated that the Ebola outbreak in Sierra Leone could have been reduced by up to a third. In treatment facilities rapid detection could have reduced health care worker infections and improved patient outcome. Reducing delays in diagnosis would result in more rapid isolation and treatment, better bed space management, and reduced nosocomial infection. Outside of treatment facilities, they would offer a diagnostic solution where none are currently available, such as determination of burial requirements or contact tracing. While LFIs can be an important part of an orthogonal system, they suffer from poor sensitivity and specificity compared to other methods. Therefore, technicians must clearly understand assay limitations and interpret the results in that context [42]. They should never be used as a stand-alone diagnostic, but when used judiciously, can improve the overall diagnostic process and outcome.

Currently, there is one LFI for LASV antigen detection. The ReLASV Rapid Diagnostic Test (RDT) is a dipstick style LFI using paired LASV NP mouse monoclonal antibodies derived from recombinant NP immunized mice. The assay was designed for detection of LASV Josiah strain in Sierra Leone [37,43–46]. Using RT-PCR as the benchmark, the LFI exhibited 91.2% sensitivity and 86.0% specificity [37]. If detection uses both the ReLASV RDT and the ReLASV antigen ELISA, the combined assays have 90.2% sensitivity and 100% specificity, again demonstrating the importance of using an orthogonal diagnostic system. Recently, an improved ReLASV Pan-Lassa Antigen Rapid Test, detecting the three most prevalent lineages of LASV (lineage II, III in Nigeria, lineage IV in Sierra Leone, Guinea, Liberia, and Mali) has been developed, but no performance date is available (https://www.zalgen.com/wp.../05/10376-ReLASV-PL-Antigen-Rapid-Test-RUO.pdf).

Magnetic bead-based assays

The MAGPIX platform (Luminex, Austin, TX, USA), based on the xMAP bead technology, employs magnetic microspheres with unique fluorescent labels resulting from different ratios of two fluorophores. Assays performed are similar to ELISA, but with a number of advantages [47•,48,49•]. The magnetic beads serve as the solid support structures for antibodies (in immunoassays) and for oligonucleotides (in molecular assays). The solution-phase kinetics of the xMAP technology ultimately results in faster assays, with higher sensitivity, and lower nonspecific binding. The ability to multiplex is one of the greatest features of the system, up to 50 discrete molecular targets can theoretically be multiplexed in a single well.

Virtually any ELISA assay can be transitioned to the MAGPIX system. For antigen detection, capture antibodies are coupled to the beads, and for antibody detection, antigens can be coupled. Satterly *et al.* compared MAGPIX and ELISA for the detection of Lassa (LASV) and Ebola virus (EBOV) specific antigen and IgM antibodies [47•]. In every format, MAGPIX was consistently more sensitive than ELISA.

The multiplex MAGPIX system is ideal for serosurveillance studies, permitting analysis multiple targets at the same time. O'Hearn *et al.* used a multiplex panel to investigate prior infections in samples collected at the Kenema Government Hospital in Sierra Leone [48]. Inactivated virus preparations were coupled to magnetic beads and reacted with samples to detect IgG antibodies to Lassa (LASV), Ebola (EBOV), Marburg (MARV), Crimean-Congo hemorrhagic fever (CCHFV), Rift Valley fever (RVFV), pan-alphavirus, and pan-flavivirus. Inactivated viruses as targets are laborious, expensive, and dangerous to produce making assay component sustainment difficult. Recombinant proteins and virus-like particles (VLPs) can be coupled to magnetic beads as a replacement for virus preparations. Ricks *et al.* used a retroviral-based VLP presenting the Venezuelan equine encephalitis virus E1/E2 glycoprotein coupled to beads to detect IgM and IgG antibodies in clinical samples with up to 2 Log_{10} sensitivity increase [49•]. Assays utilizing recombinant proteins or VLPs and monoclonal antibodies are sustainable with all components produced in bulk without biocontainment and of consistent quality.

Next generation sequencing

Next-generation sequencing (NGS) is a high throughput technology that retrieves information from the genome more quickly and inexpensively than ever before. It has revolutionized the field of biological science and enabled researchers to ask more in-depth questions of the genome. NGS utilizes metagenomics to uncover new viruses, understand diversity, monitor outbreaks in real time, and inform management decisions. Stenglein *et al.* used an unbiased metagenomic approach to discover three reptile arenaviruses (CAS virus, Golden Gate virus, and Collierville virus) as the etiologic agents for inclusion body disease in snakes [50]. Although LASV was discovered in Nigeria in 1969, NGS and molecular clocking suggest that it has existed for about 1000 years in Nigeria and between 400 to 150 years across West Africa, including Sierra Leone [20]. The fact that the Lassa virus strains in Nigeria are much older than the one circulating in Sierra Leone may have an effect on disease progression and clinical outcome of disease in each of the countries [20]. While the case fatality rate for Lassa fever in Nigeria is about 60%, it can reach 81% in Sierra Leone. A plausible explanation is that the virus in Nigeria is better adapted than the one in Sierra Leone considering the age difference. Host genetics and other factors may also explain the differences in the Lassa fever case fatality rates observed in different populations in West Africa. The genome-wide association study (GWAS) demonstrated that mutations in human gene called LARGE are associated with resistance to Lassa fever in Yoruba populations of Nigeria [51•].

Real-time NGS data have been used to support the management and control of the 2018 LASV outbreak in Nigeria by providing real-time information to the government, allaying fears arising from a purported increase in the 2018 outbreak [22••]. Data show that the 2018 outbreak was caused by multiple introductions from the rodent reservoir with very little human-to-human transmission other than nosocomial cases. The natural geography was demonstrated to be important in delineating LASV lineages in Nigeria. Sharing findings in real time with the National Centre for Disease control (NCDC) and other local health authorities aided their understanding of factors accounting for the increase in Lassa fever cases (https://ncdc.gov.ng/themes/common/docs/protocols/24_1502192155.pdf). This was made available and shared with the scientific community on virological.org (http://virological.org/t/yellow-fever-outbreak-in-nigeria-2018/274). Using NGS lead to a deeper understanding of LASV and provide answers to some difficult questions such as the origins of outbreaks, transmission, and evolution of the virus over time.

Future technology

The future of LASV diagnostics should consist of simple, robust, sensitive, and inexpensive assays that will take into consideration the genetic and geographical diversity across LASV lineages. Significant strides are being made in Lassa fever diagnostics that will continue to improve treatment, control, and prevention in endemic areas. In the near future, development of point-of-care instruments on which immunological and/or PCR-based assays can be performed will bring diagnostics to the patient for greater integration into treatment decisions and improve patient outcomes. Surveillance of LASV antibody prevalence throughout known and potential endemic geographic regions will better define the risk maps for LF. The greatest advancements in the understanding of LASV eco-epidemiology will be the result of metagenomics in outbreaks and the rodent reservoir hosts. This technology has the potential to determine unanswered questions such as the true incidence of LASV, the transmission bottleneck in LASV infection, and the virus diversity in the reservoir host. Improvements in scientific expertise throughout West Africa, using platforms like that of the African Center of Excellence for Genomics of Infectious Diseases (ACEGID), Redeemer's University, Ede, Nigeria, will build the capability to understand the disease, its control, and ultimately its prevention.

Conclusions

Our defense against emerging and re-emerging pathogens like Lassa virus, will be an improved understanding of the virus circulating in the environment, the disease in the human host, and how the virus in maintained in nature in the rodent reservoir hosts. Diagnostics are the most effective tools to meet the needs now and into the future to combat Lassa fever.

Funding

No funding was received for this work.

Conflict of interest

There are no known conflicts of interest.

Acknowledgements

This effort was funded by the Global Emerging Infections Surveillance Section of the Armed Forces Health Surveillance Branch (ProMIS plan P0141_19_RD_01) to RJS, and the Walter Reed Army Institute of Research, the Joint West Africa Research Group, and the Henry M. Jackson Foundation for the Advancement of Military Medicine via cooperative agreement W81XWH-07-2-0067 through US Army Medical Research Institute of Infectious Diseases (RJS and CTH). This work was also funded by grants from the National Institute of Allergy and Infectious Diseases, NIH-H3Africa (U01HG007480 and U54HG007480) and the World Bank (project ACE019) to CTH. Dr. Anise N. Happi, and Dr. Christian T. Happi are supported by the UK BBSRC (grant no BB/R020116/1).

Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Frame JD, Baldwin JM Jr, Gocke DJ, Troup JM: **Lassa fever, a new virus disease of man from West Africa. I. Clinical description and pathological findings**. *Am J Trop Med Hyg* 1970, **19**:670-676.

2. Buckley SM, Casals J: **Lassa fever, a new virus disease of man from West Africa. III. Isolation and characterization of the virus**. *Am J Trop Med Hyg* 1970, **19**:680-691.

3. Buckley SM, Casals J, Downs WG: **Isolation and antigenic characterization of Lassa virus**. *Nature* 1970, **227**:174.

4. Huang C, Kolokoltsova OA, Yun NE, Seregin AV, Ronca S, Koma T, Paessler S: **Highly pathogenic new world and old world human arenaviruses induce distinct interferon responses in human cells**. *J Virol* 2015, **89**:7079-7088.

5. Sarute N, Ross SR: **New world arenavirus biology**. *Annu Rev Virol* 2017, **4**:141-158.

6. Monath TP, Newhouse VF, Kemp GE, Setzer HW, Cacciapuoti A: **Lassa virus isolation from *Mastomys natalensis* rodents during an epidemic in Sierra Leone**. *Science* 1974, **185**:263-265.

7. Olayemi A, Cadar D, Magassouba N, Obadare A, Kourouma F, Oyeyiola A, Fasogbon S, Igbokwe J, Rieger T, Bockholt S *et al.*: **New hosts of the Lassa virus**. *Sci Rep* 2016, **6**:25280.

8. McCormick JB: **Lassa fever**. In *Factors in the Emergence and Control of Rodent-Borne Viral Diseases (Hantaviral and Arenal Diseases).* Edited by Saluzzo JF, Dodet B. Elsevier; 1999:177-195.

9. Fichet-Calvet E, Rogers DJ: **Risk maps of Lassa fever in West Africa**. *PLoS Negl Trop Dis* 2009, **3**:e388.

10. Yun NE, Walker DH: **Pathogenesis of Lassa fever**. *Viruses* 2012, **4**:2031-2048.

11. McCormick JB, King IJ, Webb PA, Johnson KM, O'Sullivan R, Smith ES, Trippel S, Tong TC: **A case-control study of the clinical diagnosis and course of Lassa fever**. *J Infect Dis* 1987, **155**:445-455.

12. McCormick JB, Fisher-Hoch SP: **Lassa fever**. *Curr Top Microbiol Immunol* 2002, **262**:75-109.

13. Johnson KM, McCormick JB, Webb PA, Smith ES, Elliott LH, King IJ: **Clinical virology of Lassa fever in hospitalized patients**. *J Infect Dis* 1987, **155**:456-464.

14. McCormick JB, King IJ, Webb PA, Scribner CL, Craven RB, Johnson KM, Elliott LH, Belmont-Williams R: **Lassa fever. Effective therapy with ribavirin**. *N Engl J Med* 1986, **314**:20-26.

15. Xing J, Ly H, Liang Y: **The Z proteins of pathogenic but not nonpathogenic arenaviruses inhibit RIG-I-like receptor-dependent interferon production**. *J Virol* 2015, **89**:2944-2955.

16. Paessler S, Walker DH: **Pathogenesis of the viral hemorrhagic fevers**. *Annu Rev Pathol* 2013, **8**:411-440.

17. Zinzula L, Tramontano E: **Strategies of highly pathogenic RNA viruses to block dsRNA detection by RIG-I-like receptors: hide, mask, hit**. *Antiviral Res* 2013, **100**:615-635.

18. Watanabe Y, Raghwani J, Allen JD, Seabright GE, Li S, Moser F, Huiskonen JT, Strecker T, Bowden TA, Crispin M: **Structure of the Lassa virus glycan shield provides a model for immunological resistance**. *Proc Natl Acad Sci U S A* 2018, **115**:7320-7325.

19. Bowen MD, Rollin PE, Ksiazek TG, Hustad HL, Bausch DG, Demby AH, Bajani MD, Peters CJ, Nichol ST: **Genetic diversity among Lassa virus strains**. *J Virol* 2000, **74**:6992-7004.

20. Andersen KG, Shapiro BJ, Matranga CB, Sealfon R, Lin AE, Moses LM, Folarin OA, Goba A, Odia I, Ehiane PE *et al.*: **Clinical sequencing uncovers origins and evolution of Lassa virus**. *Cell* 2015, **162**:738-750.

21. • Manning JT, Forrester N, Paessler S: **Lassa virus isolates from Mali and the Ivory Coast represent an emerging fifth lineage**. *Front Microbiol* 2015, **6**:1037.

22. •• Siddle KJ, Eromon P, Barnes KG, Mehta S, Oguzie JU, Odia I, Schaffner SF, Winnicki SM, Shah RR, Qu J *et al.*: **Genomic analysis of Lassa virus during an increase in cases in Nigeria in 2018**. *N Engl J Med* 2018, **379**:1745-1753.

23. Bausch DG, Rollin PE, Demby AH, Coulibaly M, Kanu J, Conteh AS, Wagoner KD, McMullan LK, Bowen MD, Peters CJ *et al.*: **Diagnosis and clinical virology of Lassa fever as evaluated by enzyme-linked immunosorbent assay, indirect fluorescent-antibody test, and virus isolation**. *J Clin Microbiol* 2000, **38**:2670-2677.

24. Wolcott MJ, Schoepp RJ, Norwood DA, Shoemaker DR: **Rapid infectious disease diagnostic assays**. In *Global Infectious Disease Surveillance and Detection: Assessing the Challenges—Finding Solutions, Workshop Summary.* Edited by Lemon SM, Hamburg MA, Sparling PF, Choffnes ER, Mack A. The National Academies Press; 2007:165-177.

25. Panning M, Emmerich P, Ölschläger S, Bojenko S, Koivogui L, Marx A, Lugala PC, Günther S, Bausch DG, Drosten C: **Laboratory diagnosis of Lassa fever, Liberia**. *Emerging Infectious Diseases* 2010, **vol 16**1041-1043.

26. Olschlager S, Lelke M, Emmerich P, Panning M, Drosten C, Hass M, Asogun D, Ehichioya D, Omilabu S, Gunther S: **Improved detection of Lassa virus by reverse transcription-PCR targeting the 5' region of S RNA**. *J Clin Microbiol* 2010, **48**:2009-2013.

27. Demby AH, Chamberlain J, Brown DW, Clegg CS: **Early diagnosis of Lassa fever by reverse transcription-PCR**. *J Clin Microbiol* 1994, **32**:2898-2903.

28. Lunkenheimer K, Hufert FT, Schmitz H: **Detection of Lassa virus RNA in specimens from patients with Lassa fever by using the polymerase chain reaction**. *J Clin Microbiol* 1990, **28**:2689-2692.

29. Trappier SG, Conaty AL, Farrar BB, Auperin DD, McCormick JB, Fisher-Hoch SP: **Evaluation of the polymerase chain reaction for diagnosis of Lassa virus infection**. *Am J Trop Med Hyg* 1993, **49**:214-221.

30. Vieth S, Drosten C, Lenz O, Vincent M, Omilabu S, Hass M, Becker-Ziaja B, ter Meulen J, Nichol ST, Schmitz H *et al.*: **RT-PCR assay for detection of Lassa virus and related Old World arenaviruses targeting the L gene**. *Trans R Soc Trop Med Hyg* 2007, **101**:1253-1264.

31. Trombley AR, Wachter L, Garrison J, Buckley-Beason VA, Jahrling J, Hensley LE, Schoepp RJ, Norwood DA, Goba A, Fair JN *et al.*: **Comprehensive panel of real-time TaqMan polymerase chain reaction assays for detection and absolute quantification of filoviruses, arenaviruses, and New World hantaviruses**. *Am J Trop Med Hyg* 2010, **82**:954-960.

32. Safronetz D, Lopez JE, Sogoba N, Traore SF, Raffel SJ, Fischer ER, Ebihara H, Branco L, Garry RF, Schwan TG *et al.*: **Detection of Lassa virus, Mali**. *Emerg Infect Dis* 2010, **16**:1123-1126.

33. Nikisins S, Rieger T, Patel P, Muller R, Gunther S, Niedrig M: **International external quality assessment study for molecular detection of Lassa virus**. *PLoS Negl Trop Dis* 2015, **9**:e0003793.

34. Drosten C, Gottig S, Schilling S, Asper M, Panning M, Schmitz H, Gunther S: **Rapid detection and quantification of RNA of Ebola and Marburg viruses, Lassa virus, Crimean-Congo hemorrhagic fever virus, Rift Valley fever virus, dengue virus, and yellow fever virus by real-time reverse transcription-PCR**. *J Clin Microbiol* 2002, **40**:2323-2330.

35. Ibekwe TS, Nwegbu MM, Asogun D, Adomeh DI, Okokhere PO: **The sensitivity and specificity of Lassa virus IgM by ELISA as screening tool at early phase of Lassa fever infection**. *Niger Med J* 2012, **53**:196-199.

36. Raabe V, Koehler J: **Laboratory diagnosis of Lassa fever**. *J Clin Microbiol* 2017, **55**:1629-1637.

37. Boisen ML, Hartnett JN, Shaffer JG, Goba A, Momoh M, Sandi JD, Fullah M, Nelson DKS, Bush DJ, Rowland MM *et al.*: **Field validation of recombinant antigen immunoassays for diagnosis of Lassa fever**. *Sci Rep* 2018, **8**:5939.

38. •• Gabriel M, Adomeh DI, Ehimuan J, Oyakhilome J, Omomoh EO, Ighodalo Y, Olokor T, Bonney K, Pahlmann M, Emmerich P *et al.*: **Development and evaluation of antibody-capture immunoassays for detection of Lassa virus nucleoprotein-specific immunoglobulin M and G**. *PLoS Negl Trop Dis* 2018, **12**: e0006361.

39. Wulff H, Johnson KM: **Immunoglobulin M and G responses measured by immunofluorescence in patients with Lassa or Marburg virus infections**. *Bull World Health Organ* 1979, **57**:631-635.

40. Branco LM, Grove JN, Boisen ML, Shaffer JG, Goba A, Fullah M, Momoh M, Grant DS, Garry RF: **Emerging trends in Lassa fever: redefining the role of immunoglobulin M and inflammation in diagnosing acute infection**. *Virol J* 2011, **8**:478.

41. • Nouvellet P, Garske T, Mills HL, Nedjati-Gilani G, Hinsley W, Blake IM, Van Kerkhove MD, Cori A, Dorigatti I, Jombart T *et al.*: **The role of rapid diagnostics in managing Ebola epidemics**. *Nature* 2015, **528**:S109-116.

42. Phan JC, Pettitt J, George JS, Fakoli LS 3rd, Taweh FM, Bateman SL, Bennett RS, Norris SL, Spinnler DA, Pimentel G *et al.*: **Lateral flow immunoassays for Ebola virus disease detection in Liberia**. *J Infect Dis* 2016, **214**:S222-S228.

43. Shaffer JG, Grant DS, Schieffelin JS, Boisen ML, Goba A, Hartnett JN, Levy DC, Yenni RE, Moses LM, Fullah M *et al.*: **Lassa fever in post-conflict Sierra Leone**. *PLoS Negl Trop Dis* 2014, **8**: e2748.

44. Grove JN, Branco LM, Boisen ML, Muncy IJ, Henderson LA, Schieffellin JS, Robinson JE, Bangura JJ, Fonnie M, Schoepp RJ *et al.*: **Capacity building permitting comprehensive monitoring of a severe case of Lassa hemorrhagic fever in Sierra Leone with a positive outcome: case report**. *Virol J* 2011, **8**:314.

45. Branco LM, Matschiner A, Fair JN, Goba A, Sampey DB, Ferro PJ, Cashman KA, Schoepp RJ, Tesh RB, Bausch DG *et al.*: **Bacterial-based systems for expression and purification of recombinant Lassa virus proteins of immunological relevance**. *Virol J* 2008, **5**:74.

46. Boisen ML, Schieffelin JS, Goba A, Oottamasathien D, Jones AB, Shaffer JG, Hastie KM, Hartnett JN, Momoh M, Fullah M *et al.*: **Multiple circulating infections can mimic the early stages of viral hemorrhagic fevers and possible human exposure to filoviruses in Sierra Leone prior to the 2014 outbreak**. *Viral Immunol* 2015, **28**:19-31.

47. • Satterly NG, Voorhees MA, Ames AD, Schoepp RJ: **Comparison of MagPix assays and enzyme-linked immunosorbent assay for detection of hemorrhagic fever viruses**. *J Clin Microbiol* 2017, **55**:68-78.

48. O'Hearn AE, Voorhees MA, Fetterer DP, Wauquier N, Coomber MR, Bangura J, Fair JN, Gonzalez JP, Schoepp RJ: **Serosurveillance of viral pathogens circulating in West Africa**. *Virol J* 2016, **13**:163.

49. • Ricks KM, Shoemaker CJ, Dupuy LC, Flusin O, Voorhees MA, Fulmer AN, Badger CV, Schmaljohn CS, Schoepp RJ: **Development of a bead-based immunoassay using virus-like particles for detection of alphaviral humoral response**. *J Virol Methods* 2019, **270**:12-17.

50. Stenglein MD, Sanders C, Kistler AL, Ruby JG, Franco JY, Reavill DR, Dunker F, Derisi JL: **Identification, characterization, and in vitro culture of highly divergent arenaviruses from boa constrictors and annulated tree boas: candidate etiological agents for snake inclusion body disease**. *MBio* 2012, **3**:e00180-00112.

51. • Andersen KG, Shylakhter I, Tabrizi S, Grossman SR, Happi CT, Sabeti PC: **Genome-wide scans provide evidence for positive selection of genes implicated in Lassa fever**. *Philos Trans R Soc Lond B Biol Sci* 2012, **367**:868-877.