# Field evaluation of validity and feasibility of Pan-Lassa rapid diagnostic test for Lassa fever in Abakaliki, Nigeria: a prospective diagnostic accuracy study

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## Summary

**Background** Lassa fever is a viral haemorrhagic fever with few options for diagnosis and treatment; it is also under-researched with knowledge gaps on its epidemiology. A point-of-care bedside test diagnosing Lassa fever, adhering to REASSURED criteria, is not currently available but is urgently needed in west African regions with high Lassa fever burden. We aimed to assess the validity and feasibility of a rapid diagnostic test (RDT) to confirm Lassa fever in people in Nigeria.

**Methods** We estimated the diagnostic performance of the ReLASV Pan-Lassa RDT (Zalgen Labs, Frederick, MD, USA) as a research-use-only test, compared to RT-PCR as a reference standard, in 217 participants at a federal tertiary hospital in Abakaliki, Nigeria. We recruited participants between Feb 17, 2022, and April 17, 2023. The RDT was performed using capillary blood at the patient bedside and using plasma at the laboratory. The performance of the test, based on REASSURED criteria, was assessed for user friendliness, rapidity and robustness, sensitivity, and specificity.

**Findings** Participants were aged between 0 and 85 years, with a median age of 33·0 years (IQR 22·0–44·3), and 24 participants were younger than 18 years. 107 (50%) participants were women and 109 (50%) were men; one participant had missing sex data. Although the specificity of the Pan-Lassa RDT was high (>90%), sensitivity at bedside using capillary blood was estimated as 4% (95% CI 1–14) at 15 min and 10% (3–22) at 25 min, far below the target of 90%. The laboratory-based RDT using plasma showed better sensitivity (46% [32–61] at 15 min and 50% [36–64] at 25 min) but did not reach the target sensitivity. Among the 52 PCR-positive participants with Lassa fever, positive RDT results were associated with lower cycle threshold values (glycoprotein precursor [GPC] gene mean 30·3 [SD 4·3], Large [L] gene mean 32·3 [3·7] *vs* GPC gene mean 24·5 [3·9], L gene mean 28·0 [3·6]). Personnel conducting the bedside test procedure reported being hindered by the inconvenient use of full personal protective equipment and long waiting procedures before a result could be read.

**Interpretation** The Pan-Lassa RDT is not currently recommended as a diagnostic or screening tool for suspected Lassa fever cases. Marked improvement in sensitivity and user friendliness is needed for the RDT to be adopted clinically. There remains an urgent need for better Lassa fever diagnostics to promote safety of in-hospital care and better disease outcomes in low-resource settings.

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## Introduction

Lassa fever is an infectious disease caused by the single-stranded RNA Lassa virus (LASV) of the family *Arenaviridae*.[1] LASV is transmitted mainly through contact with urine or faeces particles from, or through handling or consumption of, the small peri-domestic rodent *Mastomys natalensis*, which commonly resides among humans. Human-to-human transmission is also possible via direct contact with bodily secretions of persons infected with LASV, posing a high risk for health-care providers.[2]

Clinical diagnosis, and particularly early diagnosis, of Lassa fever is difficult due to varying presentations of the disease that mimic other endemic diseases (eg, malaria, typhoid fever, and other viral haemorrhagic fevers). When symptomatic, individuals can present with mild symptoms like fever, headache, sore throat, gastrointestinal symptoms, or general weakness. Severe disease might follow with hypotension, oedema, or respiratory distress, and might progress into a haemorrhagic phase with multiorgan failure and high mortality.[3]

Lassa fever is endemic in Nigeria, Liberia, Guinea, Sierra Leone, and other west African countries, with its endemic geographical range likely to be larger.[4] Crude estimation and underestimation of the burden of Lassa

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## Research in context

### Evidence before this study

Lassa fever presents a significant public health challenge as a zoonotic disease characterised by acute and potentially fatal haemorrhagic illness, attributed to the Lassa virus (LASV) endemic in west Africa. Despite its impact, Lassa fever remains underdetected and underdiagnosed due to non-specific symptoms that resemble those of other diseases. Molecular diagnosis through PCR serves as the reference standard, whereas alternative assays have low availability. Addressing the unmet need for point-of-care testing in Lassa fever diagnosis is crucial; however, challenges such as the diverse sequence of the Lassa virus, high-containment safety requirements, and scarcity of high-containment laboratories hinder diagnostic development and validation studies.

The ReLASV Pan-Lassa Antigen Rapid Test (Zalgen Labs, Frederick, MD, USA) is a rapid diagnostic test (RDT) that uses a mixture of polyclonal antibodies raised against recombinant nucleoproteins of representative strains from the three most prevalent LASV lineages (II, III, and IV). This dipstick-based RDT has been developed to detect nucleoproteins from fingerprick and whole-blood venipuncture specimens (ie, in whole blood, serum, and plasma) and was validated in Sierra Leone, where the LASV lineage IV (Josiah strain) is dominant. It performed well (91% sensitivity, 86% specificity) compared with its progenitor ELISA (94% sensitivity, 84% specificity; both relative to qPCR). In samples collected during the 2018 Lassa fever outbreak in Nigeria (with lineage II) when validated against Nikisins qPCR, Pan-Lassa RDT is 84·5% sensitive and 89·3% specific; compared with the Altona 1.0 qPCR (Altona, Hamburg, Germany), the Pan-Lassa RDT is 85·3% sensitive and 85·6% specific; and when compared with the combined qPCR results of both methods, for IgG and IgM seronegative samples, sensitivity was 83·3% and specificity 92·8%.

We searched PubMed, Google Scholar, and Scopus for articles published in any language from database inception to Dec 31, 2023, using the search terms "Lassa virus", "Lassa fever", "diagnostic", "rapid test", "RDT", "PCR", "sensitivity", "specificity", and "accuracy", in various combinations. From this search, as well as cross-referencing within the articles, we were able to include studies that assessed the performance of different diagnostic tools, including reviews. We found only two prospective evaluation studies on RDT for Lassa fever, one in Sierra Leone and one tested using stored samples from the 2018 Lassa fever outbreak in Nigeria. No field validation study in a routine setting for the Pan-Lassa RDT has been undertaken.

### Added value of this study

In this study, the Pan-Lassa RDT was prospectively evaluated within a routine setting in Abakaliki, Nigeria, under the REASSURED framework. Despite achieving high specificity (>90%), the RDT exhibited low sensitivity (4% at 15 min and 10% at 25 min) when using capillary blood at bedside, with upper CIs below the desired target of 90%. Sensitivity improved (42% and 50%) with laboratory-based RDT on plasma but remained inadequate. Positive RDT results correlated with lower cycle threshold values in people with Lassa fever who were PCR positive. Challenges in implementing bedside testing included cumbersome personal protective equipment and prolonged waiting periods. Our findings underscore a crucial gap in Lassa fever point-of-care testing meeting REASSURED criteria.

### Implications of all the available evidence

On the basis of the findings of this study, the current iteration of the Pan-Lassa RDT cannot be endorsed or recommended for use as a diagnostic or screening tool for suspected Lassa fever. Substantial improvements in sensitivity and user friendliness are required before considering its clinical application. The pressing need for enhanced Lassa fever diagnostics persists to ensure the safety of in-hospital care and optimise disease outcomes, especially in low-resource settings.

fever disease are attributed to challenges in surveillance and scarcity of appropriate diagnostic tests.[5,6] A widely cited, rough estimate from 1987 stands at 100 000–300 000 global incidences of Lassa fever disease leading to 5000 deaths annually.[7,8] A meta-analysis including 3063 participants from 20 west African studies[9] reported a case fatality rate of 29·6%, mostly in hospital-based settings, and an overall case fatality rate in hospital and community settings has been estimated at 1%.[7,8]

The Nigeria Centre for Disease Control and Prevention (NCDC) coordinates Lassa fever surveillance in Nigeria. From 2019 to 2022, the NCDC reported 833, 1181, 511, and 1067 respective annual confirmed Lassa fever cases, with an overall case fatality rate of 20%.[10] Most cases were reported in the states Edo and Ondo, followed by Ebonyi and Bauchi. Typically, peak incidence occurs in the dry season between epidemiological weeks 4 and 10.[11]

Early and accurate laboratory diagnosis is crucial for prompt initiation of treatments and prevention of further spread, as clinical recognition of Lassa fever can be challenging, particularly during the initial stages. This is especially important for frequently exposed health-care workers and for more vulnerable populations, such as pregnant women and unborn or young children.[12] Currently, the diagnostic method of choice for LASV is molecular diagnosis. However, this method requires advanced laboratory infrastructure, is not widely available, and does not provide immediate results. Therefore, an accurate rapid diagnostic test (RDT) that can be used at the point of care and in primary or peripheral health facilities is urgently needed. In lower-level health-care facilities, Lassa fever cases are often missed, treated wrongly, or referred late to the hospital. To date, no suitable RDT has been validated for bedside use.[13]

The ReLASV Pan-Lassa Antigen Rapid Test (Zalgen Labs, Frederick, MD, USA) is a lateral flow immunoassay RDT that detects nucleoprotein antigens of the three most prevalent LASV lineages (II, III, and IV). This RDT uses fingerprick and venepuncture specimens (whole blood, serum and plasma) and is currently in research use only.[14] A precursor version that only targeted lineage LASV IV in Sierra Leone reported 91% sensitivity and 86% specificity on serum and plasma samples compared with the Nikisins qPCR, in an epidemiological situation in which LASV lineage IV (Josiah strain) was dominant.[15] The current ReLASV Pan-Lassa RDT performed with 83% sensitivity and 93% specificity compared with two qPCR tests on stored IgM and IgG negative samples collected during the 2018 outbreak in Irrua, Edo, Nigeria, in which LASV lineage II is commonly found.[16] The latter study showed promising results for the ReLASV Pan-Lassa RDT. However, this RDT has not been evaluated in the absence of IgM and IgG immunoassay results or for its intended use as a point-of-care test that can reduce time to diagnosis. Ideally, diagnostic tests should adhere to REASSURED criteria: real-time connectivity, ease of specimen collection, affordable, sensitive, specific, user friendly, rapid, equipment free, and deliverable to end users.[17] In this study, we aimed to assess the REASSURED criteria of user friendliness, rapidity and robustness, sensitivity, and specificity of the test ReLASV Pan-Lassa RDT in a point-of-care bedside setting in Abakaliki, Nigeria.

## Methods

### Study design

In this prospective field validation study, point-of-care use of the ReLASV Pan-Lassa RDT was compared with a PCR test (Real Star Lassa Virus RT-PCR kit 2.0, Altona, Hamburg, Germany) as a reference standard. This study was conducted as part of a Médecins Sans Frontières (MSF) Operational Centre Brussels project supporting Lassa fever care in Alex Ekwueme Federal University Teaching Hospital (AE-FUTHA), Abakaliki, Ebonyi, southeast Nigeria. Ebonyi state is semirural with large agricultural areas and has around 3 million inhabitants, with approximately 675 000 living in its capital, Abakaliki. AE-FUTHA is the designated facility for Lassa fever care in the state. Adults and children with suspected Lassa fever are admitted to the facility and isolated in dedicated areas for either suspected (observation bays) or confirmed (virology unit) cases.

## Participants

The study population was recruited from people of all ages presenting at AE-FUTHA. Inclusion criteria were: admission for confirmed or suspected Lassa fever, based on the clinical criteria that were developed by MSF and AE-FUTHA (appendix 1 p 1); and written or witnessed informed consent of the participant or their guardian.

### Procedures

After obtaining written consent, trained medical staff wearing full personal protective equipment (PPE) performed the capillary blood sampling by fingerprick and performed the RDT at the patient bedside. Results were read at 15 and 25 min, aided by a visual aid chart as per manufacturer's insert;[14] RDT positivity is scored visually between 1 and 5.[14] As part of routine care, a PCR test was performed in the hospital laboratory on plasma from a venous blood sample. There, the ReLASV Pan-Lassa RDT was performed again on that same sample used for PCR (and read at 15 and 25 min). If this was not possible, a new venous blood sample was collected to be able to perform RDT in the laboratory as well as bedside.

Data collected in the wards for patients at high risk (observation bays or virology units) were scanned and sent electronically to the low-risk zone (to avoid LASV transmission). Test results and information from medical records were retrieved in the ward and entered into a standardised study form collecting age, sex, RDT results, time of diagnostic tests, cycle threshold value of the PCR assay, graduated scoring of the RDT, and batch lot of the RDT. All data were entered into a Microsoft Excel database.

Additionally, staff performing the bedside RDTs were invited to answer a short closed and open-ended survey on their perception of the use of the ReLASV Pan-Lassa RDT in clinical practice: its ease of use, interpretation, and waiting time. For additional information, the coordinating clinician (who performed most tests) was interviewed about the same topics. This latter interview provided context for the outcomes of the survey among the personnel performing the bedside RDTs.

### Diagnostic test procedures

The ReLASV Pan-Lassa Antigen Rapid Test is an RDT that uses a mixture of polyclonal antibodies raised against recombinant nucleoproteins of representative strains from the three most prevalent LASV lineages (II, III, and IV).[14] This dipstick-based RDT has been developed to detect nucleoproteins from capillary and venepuncture specimens (whole blood, serum, and plasma). The RDT was performed by trained health-care staff following the manufacturer's recommendations.[14] Visual reading was done twice for each test: at 15 and 25 min as per manufacturer's instructions (appendix 2). The bedside RDT was performed on capillary blood by medical doctors wearing full PPE who were providing direct clinical care to the patients; a second RDT was performed by the laboratory staff in the laboratory on plasma. The PCR (Real Star Lassa Virus RT-PCR kit 2.0) was used in the AE-FUTHA virology unit laboratory according to NCDC recommendations.[18]

See Online for appendix 2

Venous blood samples were collected in an EDTA tube and transferred to the laboratory, where plasma was retrieved by centrifugation. Plasma was either stored

See Online for appendix 1

| | PCR positive (N=52) | PCR negative (N=164)* | Total (N=216)* | p value |
|---|---|---|---|---|
| Sex | | | | |
| Female | 24 (46%) | 83 (51%) | 107 (50%) | .. |
| Male | 28 (54%) | 81 (49%) | 109 (50%) | 0·69† |
| Age, years | 37·5 (22·0–45·0) | 32·0 (22·8–43·3) | 33·0 (22·0–44·3) | 0·70‡ |

Data are n (%), median (IQR), or p. *One of the 165 participants with negative PCRs had missing data on sex and age. †$\chi^2$ test with Yates' continuity correction. ‡Wilcoxon rank sum test with continuity correction.

***Table 1:*** **Participant characteristics stratified by PCR result**

| | PCR positive (N=52) | PCR negative (N=165)* |
|---|---|---|
| **Bedside (cap) RDT at 15 min** | | |
| Positive | 2 (4%) | 0 |
| Negative | 47 (90%) | 147 (91%) |
| Invalid | 3 (6%) | 15 (9%) |
| **Bedside (cap) RDT at 25 min** | | |
| Positive | 5 (10%) | 0 (0%) |
| Negative | 44 (85%) | 149 (91%) |
| Invalid | 3 (6%) | 14 (9%) |
| **Laboratory (plasma) RDT at 15 min** | | |
| Positive | 24 (46%) | 7 (4%) |
| Negative | 28 (54%) | 158 (96%) |
| **Laboratory (plasma) RDT at 25 min** | | |
| Positive | 26 (50%) | 7 (4%) |
| Negative | 26 (50%) | 158 (96%) |

Data are n (%). *Three participants with a negative PCR result had missing results of the bedside (cap) RDT at 15 min, and two had missing results of the bedside (cap) RDT at 25 min. RDT=rapid diagnostic test.

***Table 2:*** **RDT test results stratified by PCR result**

at 2–8°C for 2–3 h before being processed, or frozen (–20°C) if the time to analysis would be longer than 24 h. The cycle threshold value is used to express the results of the qPCR analysis and widely recognised as inversely proportional to the viral load, with lower cycle threshold values indicating higher viral loads. In this study, a PCR test was declared positive if at least one of the two target genes (glycoprotein precursor [GPC] gene or Large [L] gene) was detected with a cycle threshold value lower than 40.

### Statistical analysis

For the RDT to be of added value in a clinical context, we targeted a sensitivity of at least 90% and specificity of at least 85%. Based on the sample size calculation (appendix 1 p 2), a minimum of 372 participants was required.

Statistical analyses were performed using R version 4.1.0. Observations with missing values were removed from analysis. Sensitivity, specificity, and positive and negative predictive values were calculated using the epiR package in R. The Clopper–Pearson 95% CIs of these values were calculated using the epi.tests function. Differences in sensitivity and specificity between bedside and laboratory RDT were tested by calculating the ratio of each classification probability between test locations along with its 97·5% CI.[19] One was added to any cell with zero counts to ensure that this ratio could be calculated. Sensitivity and specificity were compared between bedside and laboratory RDT, and would be considered different if the 97·5% CI did not include one. This calculation was carried out using the confintr package in R. The two RDT batches used in this study were compared for similarity of results (that reflected a batch-related or time-related difference). Comparisons of distribution of continuous variables were performed with a Welch two-sample *t* test or Wilcoxon rank sum test if variable distributions were non-normal. Categorical variables were compared using Pearson's $\chi^2$ test (with Yates' continuity correction) or a Fisher exact test if any cell had fewer than five observations.

This study was conducted following the principles of the Declaration of Helsinki for biomedical research involving human subjects. The study received approval by the MSF Ethics Review Board and the Ethics Committee of AE-FUTHA.

### Role of the funding source

MSF was involved in the study design, data collection and analysis, decision to publish, and preparation of the manuscript. The test manufacturer had no role in study design, data collection, data analysis, data interpretation and writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

## Results

Between Feb 17, 2022, and April 17, 2023, 217 participants were recruited for the study. Participants ranged in age from 0 to 85 years, with the median participant age 33 years (IQR 22·0–44·3), and 24 participants were younger than 18 years. Of the participants (excluding one with missing data), 107 (50%) were women and 109 (50%) were men. 52 (24%) of the 217 participants with suspected Lassa fever were confirmed positive for LASV by PCR as the reference standard. Two RDT batches were used for this study: RDT batch A (n=124) was used between Feb 17 and Dec 15, 2022, and RDT batch B (n=93) between Nov 17, 2022, and April 17, 2023. There was no evidence to show any differences in the distribution of sex and age between the participants who tested PCR positive versus negative (table 1).

The results of the RDT tests include bedside capillary blood tests at 15 and 25 min, and laboratory-based plasma tests at 15 and 25 min. Table 2 presents the results of these tests stratified by PCR result. Sensitivity, specificity, and positive and negative predictive values of the RDT performed at bedside (capillary blood) and at the laboratory (plasma) are reported in table 3. The lower 97·5% CIs for sensitivity were 0·4% for bedside RDT at

15 min and 3·4% at 25 min, and 32·2% for the laboratory RDT procedure at 15 min and 35·8% at 25 min. We did not have sufficient evidence to conclude non-inferiority in terms of sensitivity. The lower 97·5% CIs for specificity were 97·5% for bedside RDT at 15 min and 97·5% at 25 min, and 91·5% for the laboratory RDT procedure at 15 min and 91·5% at 25 min. We had sufficient evidence to conclude non-inferiority in terms of specificity. The 97·5% CIs of the ratio of sensitivities between bedside and laboratory RDT were 0·01–0·23 for 15 min and 0·03–0·36 for 25 min; as these CIs were less than 1, the laboratory RDT had higher sensitivities than the bedside RDT. There was no evidence that the sensitivity and specificity differed between RDT batches A and B (appendix 1 p 3).

Among the 52 patients with a positive PCR, PCR cycle threshold values were higher (indicating lower viral load) when laboratory (plasma) RDT results at 25 min were negative (GPC gene mean 30·3 [SD 4·3], L gene mean 32·3 [3·7]) compared with those who were RDT positive (GPC gene mean 24·5 [3·9], L gene mean 28·0 [3·6]; appendix 1 p 4). Among these cases, PCR detected both gene targets (GPC gene and L gene) in 42 instances, whereas only the GPC gene was detected in ten cases (figure). There was no evidence of correlation between RDT score and cycle threshold values of both gene target primers. The cycle threshold ranges were 19·97–36·29 (GPC gene) and 23·03–38·21 (L gene). False negative results of the bedside RDT at 15 min occurred even at the lowest cycle threshold values for both genes. Conversely, for the laboratory RDT at 25 min, only true positive results were observed up to cycle threshold values of 22·67 (GPC gene) and 27·00 (L gene), after which false negative results occurred, starting from cycle threshold values of 23·28 (GPC gene) and 27·08 (L gene).

Regarding feasibility, the tests were performed by eight different doctors on roster during the study period (Feb 17, 2022–April 17, 2023). Of the 217 bedside RDT tests that were read at 25 min, 17 (8%) had an invalid result. When stratified by personnel performing the tests, invalid test results ranged from 0% to 25% and were more common during busier hours (1200–1300 h and 1600–1800 h). The test outcomes and validity per hour and testing personnel are shown in appendix 1 (pp 5–6). Seven of the eight clinicians responded to the survey (appendix 3) for personnel performing bedside RDTs. Six of these clinicians described the ReLASV Pan-Lassa as easy to perform and to interpret. However, wearing PPE in a very hot environment made it more difficult to perform the RDT, and the different RDT scores needed careful interpretation. Three respondents expressed that the reading times of 15 and 25 min were not easily met, due to either heat while wearing the full PPE or having to wait in the high-risk zone caring for other patients who needed attention. Staying for 15–25 min with a patient with Lassa fever in the high-risk zone was perceived as inconvenient, and possibly unsafe, because LASV is "really infectious" and other tasks could not be done while staff waited.

| | Bedside (cap) RDT at 15 min | Bedside (cap) RDT at 25 min | Laboratory (plasma) RDT at 15 min | Laboratory (plasma) RDT at 25 min |
|---|---|---|---|---|
| Sensitivity | 4·1% (0·5–14·0) | 10·2% (3·4–22·2) | 46·2% (32·2–60·5) | 50·0% (35·8–64·2) |
| Specificity | 100·0% (97·5–100·0) | 100·0% (97·6–100·0) | 95·8% (91·5–98·3) | 95·8% (91·5–98·3) |
| PPV | 100·0% (15·8–100·0) | 100·0% (47·8–100·0) | 77·4% (58·9–90·4) | 78·8% (61·1–91·0) |
| NPV | 75·8% (69·1–81·6) | 77·2% (70·6–82·9) | 84·9% (79·0–89·8) | 85·9% (80·0–90·6) |

Data are % (95% CI). Invalid tests were not included. RDT=rapid diagnostic test. PPV=positive predictive value. NPV=negative predictive value.

*Table 3:* **RDT performances of different procedures**

## Discussion

A point-of-care bedside test to accurately diagnose Lassa fever is urgently needed in west African regions where health-care resources are scarce and LASV burden is high. This study evaluated the bedside performance of the ReLASV Pan-Lassa RDT with PCR as a reference standard in the referral centre for LASV of AE-FUTHA in Ebonyi, Nigeria. Although specificity was high (>95%), sensitivity of the bedside test on capillary blood was estimated at 4% (reading at 15 min) and 10% (reading at 25 min), whereas sensitivity in the laboratory on venous blood was estimated at 46% (reading at 15 min) and 50% (reading at 25 min), far below the target of 90% that was set for sensitivity. On the basis of these results, the ReLASV Pan-Lassa RDT, at its current stage of development, is not recommended as a diagnostic or screening tool for suspected Lassa fever.

A previous assessment of an earlier-generation Lassa RDT (detecting the Josiah strain, lineage IV) in Sierra Leone showed different results, with sensitivity being 91% and specificity 86%.[15] The Pan-Lassa RDT of the current study was tested in central Nigeria in 2018, where LASV lineage II was predominant, performing with 83% sensitivity and 93% specificity.[16] Our study showed much lower sensitivity of the Pan-Lassa RDT. Hereinafter, we explore several reasons for the contrasting results of the studies: sample selection for calculating sensitivity; feasibility as a point-of-care test and REASSURED criteria of user friendliness and rapidity and robustness; specimen (capillary *vs* plasma); and LASV lineage.

See Online for appendix 3

The reference standard used to analyse RDT performance was different in previously published studies. In the Sierra Leone study with the earlier-generation Lassa RDT, the authors used a composite reference standard (PCR and presence of IgM using ELISA) to assess the RDT. The study on the current Pan-Lassa RDT on samples from central Nigeria[16] was performed on PCR-positive and IgM-negative samples to select acute cases, as opposed to just PCR-positive in our study. Through the availability of the dataset (shared in the

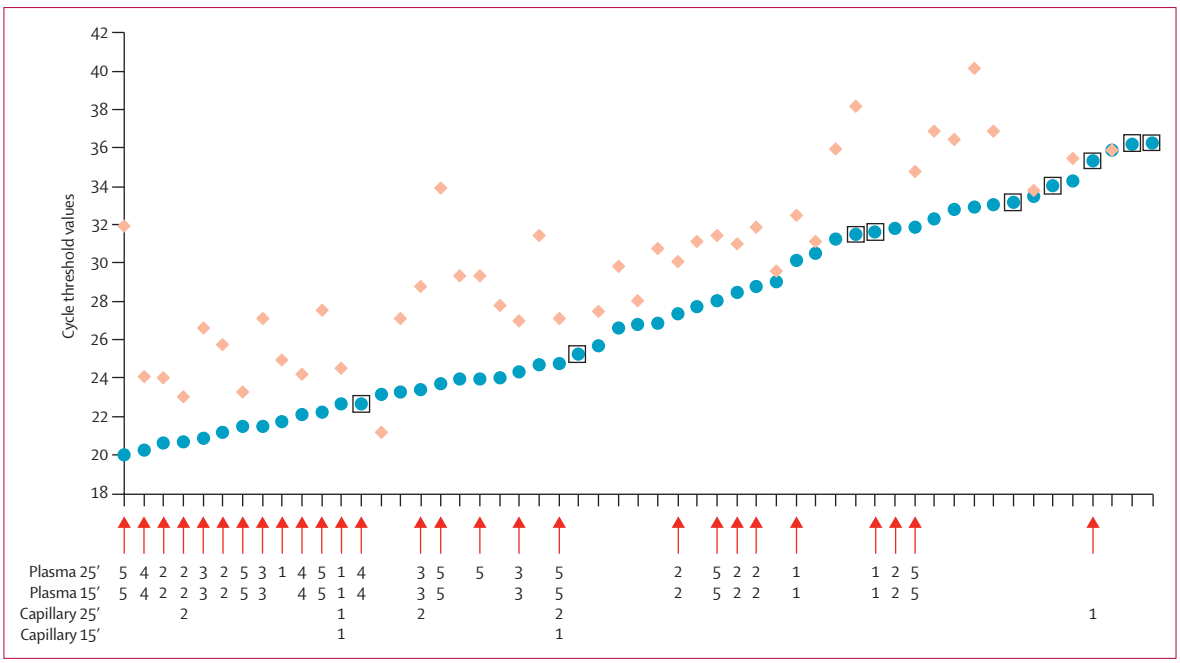


***Figure:*** **PCR cycle threshold distribution by RDT results in patients with confirmed Lassa virus**
Each line on the x-axis corresponds to a patient. Blue circles indicate GPT positive, blue circles surrounded by black squares indicate GPC positive and L negative, and orange diamonds indicate L positive. Red arrows indicate positive RDT. The values below the x-axis correspond to RDT scores (5=strong to 1=very weak) at different RDT readings. RDT=rapid diagnostic test.

supplemental material of Boisen and colleagues[16]), it was possible to recalculate the sensitivity and specificity of the RDT according to the reference standard used in our study, ie, the standard recommended by the NCDC. These calculations showed a sensitivity of 44·0% (95% CI 32·2–56·1), similar to that found in our study in the laboratory procedure (appendix 1 pp 6–7).

Although the ReLASV Pan-Lassa RDT was promoted to be suitable for point-of-care diagnosis using a fingerprick procedure,[14] its previous validations took place in laboratorial settings on stored plasma that did not represent the realities of low-resource settings where a point-of-care test is most useful.[15,16] The present study took place both in a prospective bedside and a laboratory setting and was, therefore, able to provide insights into several REASSURED criteria that were not assessed in previous retrospective studies, namely user friendliness and rapidity and robustness.[17] In the observation bays and Lassa virology unit in AE-FUTHA, patients with Lassa fever are treated by health-care staff wearing full PPE primarily to prevent nosocomial infection. Wearing PPE can be time-consuming and uncomfortable, and can complicate practical RDT procedures. This was reflected by the bedside test personnel responses to this study, who mentioned a long waiting time as a limiting factor for user friendliness of the test. Considering the negative user feedback, the poorer performance (including several invalid results) of bedside RDTs compared with laboratory RDTs (performed by experienced laboratory analysts) in the present study, and the variability of test validity between the test personnel, we cannot conclude that the Pan-Lassa RDT test is user friendly enough to be reliably performed by clinicians at bedside.

Another cause of the poorer performance of the bedside RDT compared with the laboratory RCT might be the use of capillary blood from a fingerprick for the test, instead of plasma from venous blood. This characteristic was found in previous assessments of point-of-care lateral flow tests for COVID-19 and cryptococcal antibodies, where sensitivity was lower for a fingerprick, possibly linked to volume variation of the blood inoculum or haemolysis,[20,21] although other RDTs (for hepatitis C[22] and malaria[23]) performed similarly on both sample types. It was shown that pipetting the fingerprick blood could improve sensitivity,[21] but the test is still unlikely to reach the target sensitivity of 90%. Furthermore, pipetting would be less user friendly when wearing full PPE and in low-resource settings.

The previous assessment of the Pan-Lassa RDT took place on samples from 2018, during an outbreak of LASV lineage II, sublineage g. Ebonyi state is considered an area where the same LASV lineage II circulates; it is only at the level of sublineage (a and c) that it differs.[24] Therefore, we do not expect that the difference in test performance was caused by genetic differences of the circulating LASV sublineages.

Similar to the previous assessment of the Pan-Lassa RDT,[16] we found a relation between low cycle threshold values of the PCR tests and RDT true positive

tests versus false negative tests. This relation was less apparent for the bedside RDT than for the laboratory RDT, and perhaps can be explained by the small proportion of true positive RDT results at bedside. The relation between cycle threshold value and a true positive RDT result suggests that severe disease and a higher viral load are more easily detected than less severe disease and a lower viral load.[25]

This study had several limitations. The calculated sample size was not reached, which led to substantial variance and wide CIs, for example in the positive predictive values. There could also be a small sample bias, which might influence diagnostic accuracy. However, only 90 people with LASV were registered in the region during participant recruitment,[10] 52 of whom were included in analysis. Moreover, in this preliminary analysis, we found 95% CIs that were far lower than the target sensitivity. Continuing the study would only narrow these 95% CIs but would not lead to revealing satisfactory performance of the RDT. Because the main reason for non-participation in the study was presentation of disease when medical doctors of the study were unavailable (non-differential), we suspect that differences between included and non-included participants would not have systemically affected the results. We used two RDT batches: batch A, which was received via official international order, and batch B, which was ordered nationally. We evaluated whether the two batches showed any difference in diagnostic performance, for which there was no evidence (appendix 1 p 3). Therefore, we expect this would not have biased the results. Lassa fever was diagnosed in 52 (24%) of 217 study participants. In other contexts, like community or primary health-care settings, prevalence of Lassa fever is much lower and consequently would decrease the positive predictive value and increase the negative predictive value of the ReLASV Pan-Lassa RDT. Another limitation is that we did not formally confirm or investigate the lineage or sublineage of the virus in our study; the circulating virus strain in our area is based on currently available information.

This study was a field study, mimicking routine conditions in health-care facilities that are equipped to manage Lassa fever cases (eg, adhering to PPE), which might not be the case in many peripheral centres requiring reliable point-of-care test to screen suspected cases. The strict infection prevention and control standards implemented in the study site were necessary but influenced the test perception. Although unexpected circumstances in clinical wards might have affected adherence to procedural guidelines, this is the reality of what might often happen in low-resource settings. The REASSURED criteria for user friendliness meant that "tests should be easy to perform in 2–3 steps and require minimal user training with no prior knowledge of diagnostic testing".[17] The design of this study aligned with realities in clinical centres, which might be seen as a weakness because the research did not happen under entirely controlled circumstances. However, we believe this is a strength because the outcome of the study represents both test performance and user friendliness, and field evaluation of any point-of-care test is crucial as it is the intended use of the RDT.

To our knowledge, the ReLASV Pan-Lassa RDT is the only RDT available for LASV able to detect several LASV lineages. Although it has the potential to be a valuable diagnostic tool to be used in health services at the peripheral level, in this study, overall sensitivity was too low, especially for the bedside fingerprick procedure. Therefore, in conclusion, we urgently call for available LASV diagnostic tools that can reliably screen suspected Lassa fever in low-resource settings, to promote safety of medical staff and better disease management. An antigen lateral flow test with better sensitivity, or an accessible and intuitive to perform molecular diagnostic test (eg, based on PCR or nanopore sequencing) might be developed to fill the gap in available LASV diagnostics. In the low-resource settings where LASV transmission happens, REASSURED criteria can be used as a framework for guiding the development of relevant diagnostics for LASV.

#### Contributors

TS, LdS, PFG, PC, MvH, CD-N, AA, CLO, and AO contributed to the study conceptualisation and design. TS, PC, and MvH led overall study implementation. CB and XCM were responsible for resources. HAK, CKO, NA, RE, and EOO were responsible for daily operations at the research site. JE, TS, and MvH accessed and verified the study data. CKO was responsible for laboratory analysis. JE, MvH, PC, SJ, and TS were involved in data analysis and interpretation. JE wrote the first manuscript draft, with contributions from TS, MvH, PC, and AO. All authors critically reviewed the manuscript and were responsible for the final decision to submit for publication.

#### Declaration of interests

The authors declare no competing interests.

#### Data sharing

Data are available on request in accordance with the MSF data sharing policy. Requests for access to data should be made to data.sharing@msf.org.

For the **MSF data sharing policy** see https://www.msf.org/sites/msf.org/files/msf_data_sharing_policycontact_infoannexes_final.pdf and https://journals.plos.org/plosmedicine/article?id=10.1371/journal.pmed.1001562

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