Validity and feasibility of a Pan-Lassa rapid diagnostic test for Lassa fever in Abakaliki, Nigeria: a field evaluation

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Introduction

- Lassa fever is a viral haemorrhagic fever with few options for diagnosis and treatment;
- Transmitted by rodents – *Mastomys natalensis* and by human (body fluids)
- Endemic in Nigeria, Liberia, Guinea and Sierra Leone
- 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 Nigeria

REASSURED criteria:

Real-time connectivity, ease of specimen collection, affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free or simple, and deliverable to end-users.

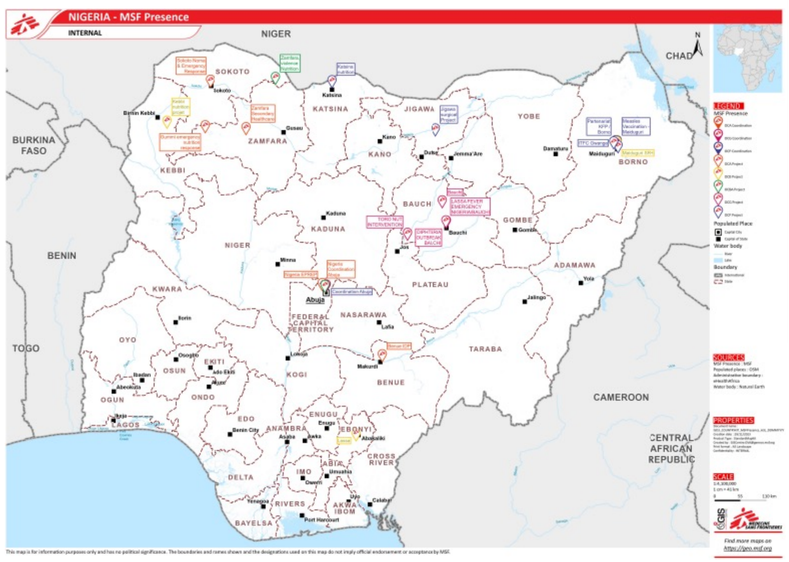
Methods

Study design: Prospective study

- **Index test**: ReLASV™ PanLassa RDT (Zalgen Labs, LCC, Germantown, MD USA 20876 and Aurora, CO, 80013, Germantown, USA US) – Research for Use Only (RUO)
- **Reference standard**: RT-PCR Altona 2.0 kit is used in AE-FUTHA VU laboratory

Setting:

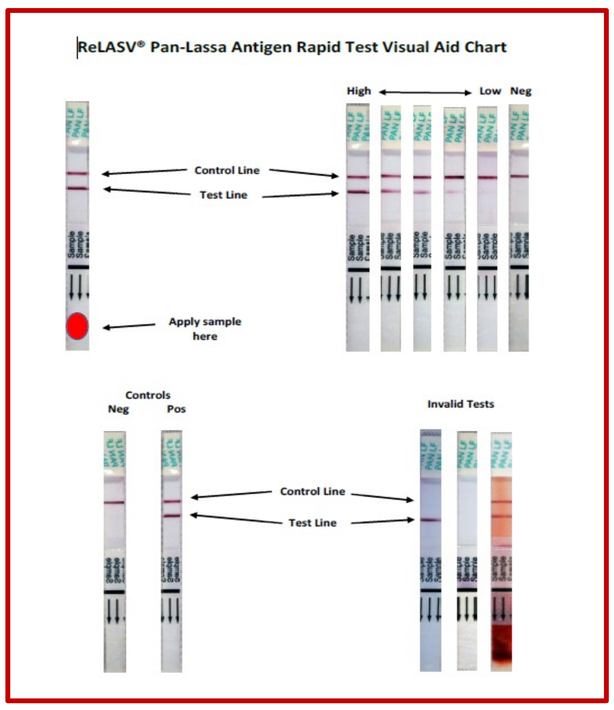
- Ebonyi state: 3 million people, 675,000 people in Abakaliki
- AE-FUTHA (Alex Ekwueme Federal Teaching Hospital, Abakaliki) is a 700 beds tertiary-level hospital
- Supported by MSF since October **2018**



Sourcegeo MSF /

Study procedure

- Sample size:
  - Minimum 340 cases, to estimated sensitivity > 90% with 95% certainty
- Patients of **all ages** with suspected Lassa fever
+ **Informed Consent**
- If yes: RDT will be performed *at bedside*, fingerprick blood
- The RDT was performed by trained health-care staff wearing full Personal Protective Equipment (PPE).
- Visual reading was done twice for each test: at 15 and 25
- Sample for RT PCR taken for routine care – with cycle threshold (Ct value) threshold of 40 to be considered positive
- GPC gene and L gene were the main primers used
- Comparison of RDT from fingerprick and venous sample (RDT repeated in the laboratory)



Source: https://zalgen.com/product/relasv-pan-lassa-antigen-rapid-test-ruo-50-test-strip-kit/

Results

- Recruitment during high season 2022-2023
- 217 participants
- Age: median 33 [22.0-44.3]
- Sex: Female: 49.5%; Male 50.5% (**Table 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. †χ^2 test with Yates' continuity correction. ‡Wilcoxon rank sum test with continuity correction.

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

Conclusions

- **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.**

	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**

- 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%. (**Table 2**)
- 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 PCR-positive participants with Lassa fever, positive RDT results were associated with lower cycle threshold values
- Personnel conducting the bedside test procedure reported being hindered by the inconvenient use of full PPE and long waiting procedures before a result could be read.

	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**

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