O.L-7 Modeling the triage of suspected Lassa fever cases using ReLASV® Antigen Rapid Test

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• Specializing diagnostics, immunotherapeutics, and novel vaccines targeting neglected topical diseases.

• Collaborations with Prof. Christian Happi of Redeemers University, Nigeria and Dr. Donald Grant of Kenema Gov’t Hospital, Sierra Leone to develop advanced Lassa fever diagnostics.

• *In vitro* diagnostics field trials at Irrua Specialist Teaching Hospital
ReLASV® *in vitro* diagnostics for LF

Zalgen has developed a suite of *in vitro* diagnostics for detection and surveillance of Lassa fever.

- ReLASV® Antigen Rapid Test for POCT
- ReLASV® Pan-Lassa Antigen ELISA for laboratory confirmation
- ReLASV® Pan-Lassa IgG/IgM ELISA for LF surveillance

The ReLASV® Antigen Rapid Test is designed as a dipstick rapid test for bedside triage of LF.

- Capable of testing fingerstick, serum, or plasma.
- Time to result: 15 min.
- Visual read – no powered equipment
- 1st Generation ReLASV® RDT Validation in Sierra Leone 95% Sensitivity, 97% Specificity
  - Reference method was combined diagnostic standard including ELISA and qPCR
  - Boisen et al., Scientific Reports (2018) 8:5939
ReLASV® Antigen Rapid Test Triage Study

Study Design:

• ISTH Lassa fever laboratory was trained in November 2017 to conduct ReLASV Dx validations.

• Retrospective testing of surplus deidentified LF case samples from Jan. – March 2018 LF case surge.

• Analysis included RDT, ELISA, qRT-PCR, outcome.
  • Subsequent Next Generation Sequencing of qPCR positive samples by ACEGID program Redeemers U. and Harvard/Broad Labs.

• Final study enrollment: 445 suspected LF cases
### LF Laboratory Screening Results

**ISTH Field Study Design: N=445 suspected Lassa fever cases**

<table>
<thead>
<tr>
<th>Test Type</th>
<th>Positive Cases</th>
<th>CFR</th>
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<tbody>
<tr>
<td>qPCR Screening (no cut-off)</td>
<td></td>
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<tr>
<td>Method R² = 0.85</td>
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<tr>
<td>Altona Positives:</td>
<td>56.4% (251/445)</td>
<td>12.4% (30/241)</td>
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<tr>
<td>Nikisins Positives:</td>
<td>49.4% (220/445)</td>
<td>16.3% (35/215)</td>
</tr>
<tr>
<td>Dual Negatives:</td>
<td>35.7% (159/445)</td>
<td>&lt;1% (1/119)</td>
</tr>
<tr>
<td>ReLASV PL RDT &amp; Ag ELISA</td>
<td>Antigen Detected: 28.3% (126/445)</td>
<td>19.2% (24/125)</td>
</tr>
<tr>
<td>ReLASV PL IgM ELISA</td>
<td>IgM Sero-Positive: 36.0% (160/445)</td>
<td>12.2% (17/139)</td>
</tr>
<tr>
<td>ReLASV PL IgG ELISA</td>
<td>IgG Sero-Positive: 32.1% (143/445)</td>
<td>9.0% (11/122)</td>
</tr>
<tr>
<td>ReLASV Dx Negative</td>
<td>PCR &amp; Dx Neg:   22.9% (102/445)</td>
<td>0% (0/76)</td>
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**Table 1. ISTH Field Trials of the ReLASV Pan-Lassa Diagnostics.**

While the samples included in this study were collected during the Jan-Mar. 2018 LF surge at ISTH the overall case fatality rate of 9.1% is lower than the NCDC reported 25% CFR nation-wide due to the inclusion of both outpatients (n=193, 3% CFR) with inpatients (n=196, 15% CFR). Rate of antigenemia (28%) compared to the rate of IgG/IgM sero-positives (43%) indicates that majority suspected case were in post-acute or early convalescent stage of LF. Approximately 9% of cases fit LF convalescent profile with IgG more reactive than IgM at >3-fold ratio.
Correlation of NGS and qPCR cycles

Figure 2 A-D. Correlation of NGS LASV Genome Assembly, RT-PCR, and Ag ELISA. A) Minimum unambiguous genome assembly length is equivalent to ≥ 4000 base pairs and corresponds to ≥ 400 reads mapped to the template genome. B) Mapped reads (≥400) correlates to RT-PCR CT = 35 cut-off. C) Logistic fit and ROC analysis using ReLASV PL Ag ELISA confirms CT = 35 cut-off for Nikisins RT-PCR method.
Correlation of qPCR and Immune response

Figure 3 A-D. Relationship of Immunoassay Reactivity and RT-PCR Testing
Key: * RDT & Ag ELISA Pos, ● RDT Pos, ○ Ag ELISA Pos, ■ IgM ELISA Pos, ♦ IgG & IgM ELISA Pos, ● IgG ELISA Pos, ♣ RDT & ELISA Neg.
ReLASV® Ag RDT Acute LF triage capability

**Detected LF Immune profiles**

- **34% (149) Acute LF - LASV Antigenemia ± IgM seropositive**
  - 102 cases confirmed by LASV qRT-PCR (based on CT cut-off)
- **29% (130) Post-Acute LF or Convalescent LF— IgG/IgM seropositive**
  - (39) Convalescent LF – IgG seropositive (qRT-PCR negative by cut-off)
- **37% (166) Non-LF by laboratory test results**

### Performance for detection of Acute LF in IgG seronegative samples.

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
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<tbody>
<tr>
<td></td>
<td>92% (55/60; CI 82 – 97)</td>
<td>87% (166/191; CI 81 – 91)</td>
<td>69% (55/80; CI 57 – 79%)</td>
<td>97% (166/171; CI 93 – 99%)</td>
</tr>
<tr>
<td>CFR</td>
<td>19% (18/93)</td>
<td>4% (7/188)</td>
<td>19% (20/104)</td>
<td>3% (5/168)</td>
</tr>
</tbody>
</table>
Comprehensive LF Diagnostic Algorithm

**LF Patient Triage Using qRT-PCR**

- **Evaluation of Major/Minor Symptoms**
- **Non Lassa Fever Symptoms**
- **Meet Lassa Fever Case Definitions**

- **N = 445**
- **16.3% CFR**

- **qPCR: 3.5hrs or more**

- **qPCR Neg**
- **qPCR Pos**

- **N = 159**
- **N = 286**

- **No CT Cut-off**

- **Confirmatory Testing:**
  - LASV Ag / IgM / IgG-capture
  - ELISA/qPCR

- **Dx Neg (63); G &/or M+ (76)**
  - CFR 4% (6/139)

- **Admit to appropriate Ward**
- **Admit to VHF Ward; Initiate ribavirin for LF per physician's order**

- **N = 159**
  - **CFR <1% (1/119)**

- **N = 145**
  - **CFR 20% (29/145)**

**LF Patient Using ReLASV Ag RDT**

- **Evaluation of Major/Minor Symptoms**
- **Non Lassa Fever Symptoms**
- **Meet Lassa Fever Case Definitions**

- **N = 445**
- **16.3% CFR**

- **RDT: <25mins**

- **RDT Neg**
- **RDT Pos**

- **N = 320**
- **N = 125**

- **Confirmatory Testing:**
  - LASV Ag / IgM / IgG-capture
  - ELISA/qPCR
  - w/ CT Cut-off

- **Dx Neg (166); G/M+ (130)**
  - CFR 4.5% (12/263)

- **Dx Pos (47)**
  - PCR/Dx Pos (102)

- **Admit to appropriate Ward**
  - **N = 296**
  - **CFR 4.5% (12/263)**

- **Admit to VHF Ward; Initiate ribavirin for LF per physician's order**
  - **N = 149**
  - **CFR 19.6% (29/148)**

**Triage of suspected LF cases using ReLASV Pan-Lassa Antigen RDT provides accurate presumptive diagnosis to aid patient management**

**How do you manage 139 patients that are Post-Acute LF or Non-LF but have been exposed to potentially infectious patients during High Containment Hold?**
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