Establishment of diagnostic tools for detection of Lassa virus & experience from the Irrua Specialist Teaching Hospital, Edo State, Nigeria

Irrua Specialist Teaching Hospital - ISTH
Prof. Sylvanus Okogbenin - CMD
Dr. Danny Asogun
Dr. Ephraim Ogbaini-Emovon (ILFRC coordinator)
Prof. George Akpede

Bernhad-Nocht Institute for Tropical Medicine
Prof. Stephan Günther
Institute of Lassa Fever Research and Control (ILFRC)

DIAGNOSTICS
2008

Irrua Specialist Teaching Hospital
Work load in ISTH Lassa diagnostic lab

- **2008 – 2018**
  - >13 000 samples
  - 1 650 LASV positive
- **5 September 2018 (2018)**
  - 2 466 suspected cases tested
  - 497 LASV positive
- **91% done @ISTH**

Origin of LASV positive diagnostic requests 2009-2016
Total: 1051 – unknown: 277

LASV: Lassa virus
LASF: Lassa fever
Conventional and real time RT-PCR

- Separated rooms-diagnostic workflow
- 24 laboratory staff trained by BNITM since 2008
- Rooster including nights and Weekends
- 2-4 visits/year of BNITM staff
ISTH diagnostic laboratory 2008-2018

Reception → Extraction
Inactivation → Master Mix
Amplification → Results
Molecular diagnostics of LASV
RT-PCR

• 2008-2017
  Conventional RT-PCR:
    ➢ In-house S segment

• 2017-ongoing
  Real-time RT-PCR:
    ➢ Altona S segment
    ➢ Nikisins L segment
Lassa virus Real-time RT-PCR

Virus variability poses problem to probe and primer design

- Reduced contamination risk compared to conventional PCR
- Short turn around time (~ 5hr)
- Quantitative read-out (Cycle threshold)
- Internal process control (inhibition/sample quality)
- Industry standard and quality (Altona)
2017: Lassa virus Real-time RT-PCR

Virus variability poses problem to probe and primer design

Real-time RT-PCR assays

• Real-time RealStar® Lassa Virus RT-PCR Kit 1.0 targeting the S segment

• Real-time RT-PCR assay based on primers and probes targeting the L segment as published in Nikisins et al. 2015
Development and validation of LASV RT-PCR

ISTH

First pre-evaluation of Altona 1.0 kit (500 samples)

Clinical evaluation of Altona 1.0 kit and Nikisins (850 samples)

First pre-evaluation of 2.0 kit (200 samples)

Clinical evaluation of 2.0 kit in Nigeria (>800 samples)


BNITM

Improvement of Altona 1.0 kit

Optimisation of Nikisins PCR

Reactivity and specificity testing of Altona 1.0 kit and Nikisins

Improvement of 2.0 kit

Reactivity and specificity testing of 2.0 kit, clinical evaluation (200 samples)

Diagnostic at ISTH

Conventional (GPC, gel-based)

Altona 1.0 (GPC) & Nikisins (L)

Altona 2.0 (GPC and L)

Europe

Lab evaluation of 2.0 kit in 20 different labs (EMERGE)
RealStar® Lassa Virus RT-PCR Kit 1.0 CE

The RealStar® Lassa Virus RT-PCR Kit 1.0 is an in vitro diagnostic test, based on real-time PCR technology, for the qualitative detection of Lassa virus (LASV) specific RNA.
Target S Segment

Lassa

Internal Control
Reactivity Altona 1.0 and Nikisins using isolates in BSL-4 (CDC Atlanta and BNITM)

- **169** LASV isolates
  - 38 CDC & 131 BNITM
  - from Nigeria, Benin, Togo, Côte d'Ivoire, Guinea, Liberia, and Sierra Leone
  - Representing all known genetic lineages (I-VI) collected between 1969 and 2017

- Both assays detected all strains, although with different efficacy
  - Ct difference up to 17 between lineage II (Nigeria) and VI (Togo)

- No cross-reactivity after testing of 34 different viruses (e.g. Ebola, RVFV, Dengue, YFV, HCV) as well as Malaria
Clinical accuracy testing in Nigeria (2017)

- Sensitivity and specificity of the Altona 1.0 and Nikisins were estimated using
  - plasma samples collected from 803 patients suspected to have LASF in Nigeria.
  - Conventional gel-based RT-PCR (Ölschläger et al. 2010) used as a reference test
## Clinical accuracy testing in Nigeria (2017)

<table>
<thead>
<tr>
<th></th>
<th>Conventional RT-PCR assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>RealStar real-time RT-PCR assay</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>Performance</td>
<td>Sensitivity 96.5% (92.1-98.5%)</td>
</tr>
<tr>
<td>Nikisins real-time RT-PCR assay</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>Performance</td>
<td>Sensitivity 88.3% (82.1-92.6%)</td>
</tr>
<tr>
<td>RealStar and Nikisins real-time RT-PCR assays combined</td>
<td>Positive*</td>
</tr>
<tr>
<td></td>
<td>Negative**</td>
</tr>
<tr>
<td>Performance</td>
<td>Sensitivity 98.6% (95.0-99.6%)</td>
</tr>
</tbody>
</table>

*One or both of the assays are positive.

**Both assays are negative.
Both assays must be used in combination to ensure reliable detection of all Lassa virus lineages and strains.
Next generation of kit
2018 - Validation of Altona 2.0 kit

DUO kit with the two assays: 1 kit enables testing of
• S (Altona 1.0) &
• L segments (Altona improved the Nikisins PCR)
Development and validation of LASV RT-PCR – 2.0 kit

First pre-evaluation of Altona 1.0 kit (500 samples)

Clinical evaluation of Altona 1.0 kit and Nikisins (850 samples)

First pre-evaluation of 2.0 kit (200 samples)

Clinical evaluation of 2.0 kit in Nigeria (>800 samples)


Improvement of Altona 1.0 kit

Optimisation of Nikisins PCR

Reactivity and specificity testing of Altona 1.0 kit and Nikisins

Improvement of 2.0 kit

Reactivity and specificity testing of 2.0 kit, clinical evaluation (200 samples)

Lab evaluation of 2.0 kit in 20 different labs (EMERGE)

Diagnostic at ISTH

Conventional (GPC, gel-based)

Altona 1.0 (GPC) & Nikisins (L)

Altona 2.0 (GPC and L)
Next generation of kit
2018 - Validation of Altona 2.0 kit

Started on 15 October 2018 in Nigeria and Europe

- 280 samples tested so far
- Ongoing
„Gold Standard“: IgG and IgM Immunofluorescence with LASV infected cells (produced in BSL4)
Antibody detection
Indirect immunofluorescence

Disadvantage: Antibodies rise late in infection
Advantage: Retrospective diagnosis possible

Virus infected Cell

- Fluorescence dye
- anti-IgG/IgM
- Patient antibodies
- Virus antigen
ELISA development and evaluation – BNITM experience

- LASV antigen: recombinant nucleoprotein (NP)
  - IgM ELISA
  - IgG ELISA

- Analytical and clinical evaluation was performed with 880 sera from LASF endemic (Nigeria) and non-endemic (Ghana and Germany) areas.

Gabriel et al. 2018
ELISA development and evaluation – BNITM experience

Clinical performance characteristics of IgM ELISA as stand-alone test and in combination with IgG ELISA for early diagnosis of LASF

**Using RF or CD32 IgG ELISA as reference method**

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM</td>
<td>31.1%</td>
<td>95.7%</td>
</tr>
<tr>
<td>IgM &amp; IgG</td>
<td>25.9%</td>
<td>100%</td>
</tr>
</tbody>
</table>

**Using IFA as reference method**

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM</td>
<td>96%</td>
<td>84.3%</td>
</tr>
<tr>
<td>IgM &amp; IgG</td>
<td>95.4%</td>
<td>90.1%</td>
</tr>
</tbody>
</table>
Deployment of ELISA developed in a pilot epidemiological study (July – October 2018)

Seroprevalence, Incidence and Risk Factors of Lassa Fever in Edo State, Nigeria - a Pilot Study.

- 700 participants from urban and rural endemic areas enrolled.
- Samples and data to be analysed Nov/Dec 2018
- Baseline IgM and IgG levels to be determined
Conclusions for ELISA

• ELISA are not equivalent to RT-PCR for early diagnosis of LASF
  – Of value in diagnosing patients at later stage of disease
• Isolated IgM reactivity
  – IgM, in the absence of IgG, has little diagnostic value
• IgG ELISA for epidemiological studies and clinical trials
  – high specificity
  – higher throughput rate and easier set-up compared to IFA
• Commercialisation of LASV ELISA IgM and IgG kits by BNITM pending
• Limitations: Evaluation needed in other endemic regions
Conclusions for LASV diagnostics and surveillance

- Real-time RT-PCR (2 targets)
- IFA based on infected cells
- ELISA based on infected cells
- ELISA based on recombinant NP
- ELISA based on recombinant GP
- ELISPOT to be developed
- Neutralization assay (BSL-4 BNITM protocol developed)
- Antigen testing
Practical considerations

• Preferred specimen type, handling and inactivation
  – RT-PCR: EDTA blood, inactivation with AVL buffer
  – ELISA: Dry tube or EDTA, serum diluted 1:20 in 1% Triton X-100–PBS

• Biosafety for molecular and serological assays:
  – Glovebox preferred for first inactivation step

• Provision of reference materials
  – European Virus Archive, EVAg

• Availability of international proficiency panels
  – EU consortia EMERGE, others
Development of national testing algorithm

RT-PCR

• Laboratory meeting in Abuja, 26-27 March 2018
  – Irrua Specialist Teaching Hospital (ISTH)
  – Lagos University Teaching Hospital
  – National Reference laboratory, Gaduwa
  – Federal Teaching Hospital Abakaliki (FETHA)
  – ACEGID/Redeemer’s University
  – Nigerian Ministry of Defence Reference Laboratory
  – International partners: BNITM, US CDC, PHE, WHO
Draft of national testing algorithm

RT-PCR

• Recommendations for specimen types:
  – Whole blood in EDTA (a minimum volume of 4mL);
    • 5ml from adults,
    • 2-3mls from children,
    • 1.5ml from neonates

• 2 assays, run in parallel
  • Altona 1.0; S segment
  • Nikisins assay; L segment

• Currently adopted by 3 labs (ISTH, NRL, FETHA)
Experience with current molecular diagnostics

• Challenges with international procurement and delivery into Nigeria

• Testing methodology requires continuous training and TA to new labs

• Large investment in infrastructure: power, laboratory equipment...

• Sample management and transportation logistics has improved since early 2018
  – National courier being used; TRANEX
Next steps to improve diagnostics

• Collaboration with WHO and FIND through R&D Blueprint
  – Strengthening national laboratory capacity
  – Development of new diagnostics
  – Support accreditation of clinical trial laboratories
  – Support development of a national sample archive
• Support for procurement and training in 2018
• Awaiting ERD approval for Altona 2.0
<table>
<thead>
<tr>
<th>INTENDED USE CASE</th>
<th>TARGET USE SETTING/DIAGNOSTIC NEEDS</th>
<th>4.</th>
<th>3.</th>
<th>2.</th>
<th>1.</th>
<th>0.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>National reference lab</td>
<td>MDx*, † POC MDx *, † IA *, †</td>
<td>MDx*, † POC MDx <em>, † IA</em>, †</td>
<td>POC MDx * POC IA* RDT *</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case detection, management</td>
<td>Referral/ regional hospital</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>District hospitals</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Health centres</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Health posts, field settings</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surveillance</td>
<td>Referral/ regional hospital</td>
<td>MDx* sequencing IA *, †</td>
<td>MDx* POC MDx * IA *</td>
<td>POC MDx * POC IA* RDT *</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Health centres</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outbreak response</td>
<td>Referral/ regional hospital</td>
<td>MDx* sequencing IA *, †</td>
<td>MDx* POC MDx * IA *</td>
<td>POC MDx * POC IA* RDT *</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Health centres</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical trials</td>
<td>Health centres</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(vaccine, therapeutics)</td>
<td>Health posts, field settings</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

POC, point of care or near-patient; MDx, molecular diagnostic (e.g. reverse transcriptase [RT]-PCR); IA, immunoassay (e.g. ELISA); RDT, rapid diagnostic test (e.g. lateral flow immunoassay); VHF, viral haemorrhagic fever. * Requires pan-LASV detection (Devy et al., 2018 manuscript accepted, BMJ Global health)
Molecular epidemiology at the epicentre of the 2018 outbreak: ISTH

- 7 weeks metagenomic sequencing efforts @ISTH
- 120 samples sequenced @ISTH
- 36 analyzed @ISTH

Transmission: rodent spill-over
Irrua Specialist Teaching Hospital - ISTH

Bernhad-Nocht Institute for Tropical Medicine
- Dr. Meike Pahlmann
- Dr. Lisa Oestereich
- Dr. Sophie Duraffour

BNITM
Bernhard-Nocht-Institut für Tropenmedizin

HARVARD

GHP
Programme

World Health Organization

DFG
Deutsche Forschungsgemeinschaft
German Research Foundation