Abstract

Nucleic acid testing (NAT) methods are now considered to be gold standard for detection of pathogens. These methods allow higher sensitivity and specificity compared to alternatives based on antibodies and culturing. The requirement of expensive equipment, specialized laboratories, and trained personnel precludes routine NAT in most of the developing world and even in resource limited parts of the United States. The ongoing outbreak of Ebola virus (EBOV) in West Africa has highlighted the need for rapid molecular diagnostic tests which can be used at point-of-care (POC) with limited training and simple equipment. Here we report on development of a simple, sensitive, and easy to use diagnostic test for detection of EBOV at POC.

This assay is based on Loop-mediated isothermal AMPlification (LAMP) of the RNA viral genome of the current EBOV-Zaire strain. Analytical sensitivity was determined by testing 10-fold serial dilutions of RNA extracted from Ebola virus – Zaire, Kikwit (1995) and strain from the ongoing outbreak (Guinea-2014) in W. Africa. A true POC test for Ebola was developed, consisting of lyophilized amplification reagents, a rapid one step sample preparation method, and a battery operated isothermal instrument capable of detecting a fluorescent signal in positive reactions.

Results

A) Isothermal Detection of EBOV
• LAMP primers were designed targeting a conserved region of the spike glycoprotein (GP) gene.
• Amplification was performed using OmniAmp™ DNA polymerase.
• Reaction temperature optimal of 72°C was obtained by thermal cycler gradient profile.
• Real time monitoring of amplification by using DNA intercalating dye in the amplification mixture.
• Assay time: 40 min.

Table 1: Sensitivity of EBOV LAMP assay

<table>
<thead>
<tr>
<th>Target</th>
<th>Time to Result (min) Std. Dev.</th>
<th>RNA copies per rxn.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zaire – Kikwit (1995)</td>
<td>16.6 0.46</td>
<td>7 x 10^4</td>
</tr>
<tr>
<td></td>
<td>14.5 0.18</td>
<td>7 x 10^7</td>
</tr>
<tr>
<td></td>
<td>16.5 1.18</td>
<td>7 x 10^6</td>
</tr>
<tr>
<td></td>
<td>19.4 0.10</td>
<td>7 x 10^5</td>
</tr>
<tr>
<td></td>
<td>25.5 1.13</td>
<td>1.4 x 10^5</td>
</tr>
<tr>
<td></td>
<td>26.9 0.33</td>
<td>7 x 10^4</td>
</tr>
<tr>
<td></td>
<td>30.4 4.41</td>
<td>7 x 10^3</td>
</tr>
<tr>
<td>NTC</td>
<td>48.7 4.46</td>
<td>NTC</td>
</tr>
</tbody>
</table>

B) Assay specificity
• Specificity was determined by testing RNA extracts from closely related viruses: Lassa Fever virus (LV)- Josiah strain, Marburg virus (MARV)- Musoke strain, Chikungunya virus (CHIKV), Crimean–Congo hemorrhagic fever virus (CCHFv), West Nile virus (WNV), and Dengue fever virus (DENV) serotypes 1–4.

C) EBOV LAMP using dried reagents
• Amplification reagents were lyophilized to ensure long term stability of LAMP reagents for use in field conditions without maintaining cold chain.
• Complete formulation (master mix, LAMP primers, and dye) were added to 0.2 ml PCR tubes, and dried overnight in a lyophilizer.
• After lyophilization, tubes were stored at room temperature in a pouch with a desiccant.
• To test performance of lyophilized reagents, 25 µl of 10-fold dilution of target was added to dried reagents and tubes were incubated at 72°C.

Figure 1: Specificity of EBOV LAMP assay.

D) Instrumentation
• Required instrumentation: Heat block (for heat lysis) and a isothermal block (AmpliFire™, Douglas Scientific, MN).
• Features of AmpliFire™:
  - Light weight (< 5 lbs.) and portable
  - Small footprint and battery operated
  - 8- wells
  - Touch-screen user interface for easy operation,
  - Real time monitoring of amplification reaction
• Results are displayed on the screen as “positive” or “negative”
• Data can be stored on the device itself or transferred using a USB port.

Figure 3: AmpliFire™

E) Workflow for POC EBOV LAMP test:

Suggested Workflow

1. Place sample into 2 ml tube
2. Place 2 ml tube containing LAMP mix into AmpliFire™
3. Incubate at 72°C for 30 min
4. Read result

Figure 2: Sensitivity of EBOV LAMP using dried reagents.

Conclusions

• EBOV LAMP diagnostic test with results available in under 40 minutes.
• High sensitivity (2800 cp/rxn) for current outbreak strain.
• High specificity, no amplification of any other viral targets.
• Simple, rapid sample preparation step (5 min.
• Dried reagents for room temperature storage.
• Simple and easy to use workflow.
• Suitable for POC use, no need of extensive training or expensive instrumentation.
• Further work is underway to develop the test and submit for Emergency Use Authorization in 2015.

Acknowledgement

This work was supported by grant from NIAID to Dr. Yogesh Chander.