Note

Rapid molecular diagnostic test for Zika virus with low demands on sample preparation and instrumentation

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Abstract

Zika virus has only recently gained attention due to recent large outbreaks worldwide. An easy to use nucleic acid amplification test could play an important role in the early detection of the infection and patient management. Here, we report a rapid and robust isothermal nucleic acid amplification assay for the detection of Zika virus. The method is cost-effective and compatible with portable instrumentation, enabling near patient testing and field use.

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1. Report

The recent Zika virus outbreak sparked renewed research interest in virus detection (Rubin et al., 2016). There is increasing evidence that Zika virus increases the risk of microcephaly, a major birth defect, and Guillain–Barré syndrome in infected individuals (Rubin et al., 2016; Chang et al., 2016; Driggers et al.). The established transmission route is via an insect vector, mainly the Aedes aegypti mosquito, while sexual transmission has been demonstrated (Chang et al., 2016; Zanluca & dos Santos, 2016). Diagnosis of Zika virus has so far relied on serological methods which are often time consuming or nucleic acid amplification tests (NAATs) such as real-time reverse transcription polymerase chain reaction (RT-PCR) (Faye et al., 2008, 2013; Balm et al., 2012). RT-PCR requires the use of high precision instruments for thermal cycling reactions and skilled personnel for performing the complex protocol and data interpretation. Consequently, RT-PCR has been primarily confined to specialized large central laboratories.

NAATs performed on relatively simple, portable, and low-cost devices are becoming increasingly desirable as they can be employed in field testing or point-of-care settings, allowing for a rapid response to outbreaks particularly in low resource settings. Here, we present an alternative NAAT, the reverse transcription strand invasion based amplification (RT-SIBA) assay for the rapid detection of Zika virus.

RT-SIBA has been previously applied to the rapid detection of DNA (Hoser et al., 2014; Eboigbodin & Hoser, 2016) and RNA (Eboigbodin et al., 2016) from pathogens. During RT-SIBA reactions, Zika virus RNA is first reverse transcribed to cDNA followed by amplification and detection of cDNA under isothermal reaction conditions. SIBA relies on a recombinase-coated single-stranded invasion oligonucleotide (IO) for the separation of a complementary target duplex. This results in the generation of a single-stranded target template that is bound and extended by target-specific primers via DNA polymerase. The repeated cycles of strand separation and primer extension of the target lead to an exponential amplification at low and constant temperature. The method can be run on relatively low-cost devices and features low sample preparation, and has the potential to allow for the early detection of outbreaks, treatment management, and preventing the spread of the virus. We also compared the performance of RT-SIBA with real-time PCR for the detection of Zika virus.

Zika virus genome sequences were retrieved from the GenBank sequence database and aligned for identification of conserved regions. RT-SIBA and RT-PCR assays were designed to detect the 5′ conserved region of the Zika virus genome. However, protocol and material. Both RT-SIBA and RT-PCR reactions were detected using the intercalating dye Sybr Green 1. The analytical sensitivities of both the RT-SIBA and RT-PCR Zika virus assays were established in at least three independent experiments using serial dilutions of Zika virus strain MR766 (a kind gift from Dr. Livia Schrick, Robert Koch Institute, Germany). The in vitro transcribed RNA was used at 10^7 copies.

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Sensitivity of the Zika virus RT-SIBA assay compared with the RT-PCR assay.*

<table>
<thead>
<tr>
<th>Zika virus transcript copy number per reaction</th>
<th>Average time to positive results (min) RT-SIBA</th>
<th>Average time to positive results (min)* (Threshold cycle, Ct) RT-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>10^2</td>
<td>10.3</td>
<td>50.3 (13.8)</td>
</tr>
<tr>
<td>10^3</td>
<td>11.4</td>
<td>54.8 (17.4)</td>
</tr>
<tr>
<td>10^4</td>
<td>12.9</td>
<td>61 (22.4)</td>
</tr>
<tr>
<td>10^5</td>
<td>14.7</td>
<td>64.4 (25.1)</td>
</tr>
<tr>
<td>10^6</td>
<td>17.1</td>
<td>68.4 (28.3)</td>
</tr>
<tr>
<td>10^7</td>
<td>18.5</td>
<td>71.9 (31.1)</td>
</tr>
<tr>
<td>10^8</td>
<td>25.8</td>
<td>74.1 (32.9)</td>
</tr>
<tr>
<td>0</td>
<td>ND</td>
<td>74.8 (33.4)</td>
</tr>
</tbody>
</table>

* The detection time for RT-PCR was determined according to the threshold cycle (Ct) value. This was calculated directly from the One-step PCR program without taking into account the ramping time required for thermal cycling. ND, not determined; RT-PCR, real-time reverse transcription polymerase chain reaction; RT-SIBA, reverse transcription Strand Invasion Based Amplification. The results are expressed in copy number per reaction.

RT-SIBA did not produce any detectable signals in the absence of a template, whereas a detectable signal was produced by RT-PCR after 30 threshold cycles (Ct). The Ct values for the no-template controls (NTC) were similar or overlapped with the Ct values for low copies of \textit{in vitro} transcribed Zika virus RNA. This is likely due to primer dimers as the reactions were performed using an intercalating dye rather than a target-specific probe (Chou et al., 1992). The use of target specific probes such as Taqman and molecular beacons eliminates the detection of primer dimers. The specificities of both amplification methods for the detection of Zika virus were established by challenging the reaction with 10^2 copies of RNA from yellow fever, dengue 1, West Nile and Chikungunya virus. The assays were further challenged by the addition of DNA pooled from 15 different unrelated microorganisms (10^3 DNA copies per reaction; see Supplementary material for the list of unrelated microorganisms). Yellow fever, dengue 1, West Nile, Chikungunya virus, or any of the 15 different unrelated microorganisms were not detected by the RT-SIBA and RT-PCR Zika assays. This may indicate that both the RT-SIBA and RT-PCR assays are specific for the detection of Zika virus RNA.

The performance of RT-SIBA in the detection of Zika virus was further determined using the commercially available NATtrol Zika Virus Range Verification Panel samples (ZeptoMetrix, Buffalo, NY, USA) that were developed to mimic clinical specimens with Zika virus infection. The panel consisted of three Zika virus specimens that differed in their viral load (high, medium, and low) and a negative specimen. Each specimen was subjected to quick crude lysis by the addition of 10−50 μl of lysis buffer (2% Triton X-100, 12 mM magnesium acetate), and 2 μl of this mixture was added to the reaction. The RT-SIBA Zika virus assay was observed to detect all three Zika virus specimens, with no cross-reaction with the negative specimen. The average time to positive results for clinical specimens with high, medium, and low Zika viral loads was 18, 20, and 22 minutes, respectively (Fig. 2A). This suggests that RT-SIBA does not require highly purified RNA for performing the
reactions. Similar reactions were performed on a battery-operated portable fluorescence detection system (Orion GenRead, Orion Diagnostica Oy, Espoo, Finland), since RT-SIBA is performed at a low and constant temperature (Fig. 2B). The portable fluorescence detection system was found to perform similarly to the qPCR device, highlighting the suitability of RT-SIBA for field applications. Despite the rapid detection displayed by RT-SIBA Zika virus assay, the assay still needs to be fully validated for direct detection of clinical specimens infected with Zika virus. The rapid detection and high analytical sensitivity displayed by RT-SIBA for the detection of Zika virus, as well as tolerance to sample-derived inhibition, demonstrate that the method may be a powerful molecular diagnostic tool for the detection of Zika virus. Since the method can be run on portable and relatively low-cost devices, it may be applied in the rapid response to outbreaks.

Authors Contributions

KE conceived the study, KE, TO and MB designed the experiments, KE and TO performed the experiments, KE, TO, MB and MH analyzed results, KE TO MB MH wrote the manuscript.

Competing Interest

KE TO MB employees of Orion Diagnostica Oy. All SIBA patents/patent applications are owned by Orion Diagnostica Oy. MH has no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.diagmicrobio.2016.08.027.

References


