RECOMBINASE POLYMERASE AMPLIFICATION-BASED ASSAYS TO DIAGNOSE SOIL-TRANSMITTED HELMINTHS IN STOOL

Jason L. Cantera, Heather White, Helen Storey, Tala de los Santos

BACKGROUND

Soil-transmitted helminths (STH)

STH are parasitic intestinal worms that affect up to 2 billion people worldwide. STH can be controlled with mass drug administration (MDA) for at-risk populations. Infection levels must be closely monitored to assess the impact of MDA and guide control programs. Current copromicroscopy-based diagnostics for STH are labor-intensive to perform in the field and lack sensitivity to detect low-intensity infections, which are more common after repeated MDA. There is a need for diagnostic tools with improved performance as compared to current methods to support late-stage control program decisions, such as informing when to stop MDA and for post-MDA surveillance.

Recombinase polymerase amplification (RPA)

RPA is a field-appropriate isothermal nucleic acid amplification technique that offers rapid amplification times with potential for simplified instrumentation due to its low temperature requirement. PATH is developing new molecular diagnostics for STH (Ascaris lumbricoides, Trichuris trichiura, Necator americanus, and Ancylostoma duodenale) based on RPA technology.

MATERIALS AND METHODS

- Gene targets, plasmid control, and oligonucleotides. Target genes for qPCR assays were used as RPA targets for STH. The STH target regions were cloned in plasmids for use as positive controls. Primers and probes were designed based on TwistAmp™ reaction kit manual, and purchased from either Integrated DNA Technologies or Biosearch Technologies.
- Specimens, DNA extraction, and PCR. Stool specimens were characterized by Kato-Katz. Extracted genomic DNA from stool was characterized by real-time PCR. Primers were designed and screened using either TwistAmp basic or exo kit and plasmid DNA. Reactions were prepared and optimized according to manufacturer instructions. Amplifications was observed by using either gel electrophoresis (basic), or fluorescence detection (exo) and read by Twista® fluorometer.
- Performance evaluation. Analytical sensitivity/limit of detection (LOD) was determined using a series of plasmid dilutions (10^2 to 1 copy). Specificity was determined using DNA from stools that were either uninfected, STH-infected, or infected with other parasites based on Kato-Katz (KK) or real-time PCR analysis.

RESULTS

Analytical sensitivity of Ascaris RPA

The assay had a limit of detection of 10 plasmid copies with intermittent detection to as low as 3 copies. This was equivalent to testing the same panel with PCR.

RESULTS CONTINUED

Specificity of Ascaris RPA and tolerance to background DNA

The assay amplified only from Ascaris-positive stools (based on KK and PCR); no amplification of hookworm and Trichuris in negative control.

DNA background inhibition was determined using 10 plasmid copies plus amounts of DNA extracted from stool. The rate of amplification was unaffected by increasing amounts of DNA.

Performance of the RPA assays versus PCR

<table>
<thead>
<tr>
<th>Species-specific RPA assay</th>
<th>Target gene</th>
<th>Detection limit (copies)</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. lumbricoides</td>
<td>ITS1</td>
<td>&lt;10</td>
<td>Hk+ Asc+ Tri+</td>
</tr>
<tr>
<td>T. trichiura</td>
<td>18S</td>
<td>&lt;20</td>
<td>- - +</td>
</tr>
<tr>
<td>An. duodanale</td>
<td>ITS2</td>
<td>&lt;10</td>
<td>+ - -</td>
</tr>
<tr>
<td>N. americanus</td>
<td>ITS2</td>
<td>&lt;10</td>
<td>+ - -</td>
</tr>
<tr>
<td>B. subtilis (IAC)</td>
<td>ropB</td>
<td>100</td>
<td>- - -</td>
</tr>
</tbody>
</table>

Each RPA assay consistently detected 10-20 plasmid copies (14/14 replicates), suggesting high sensitivity. LOD of B. subtilis (as IAC) RPA assay was 100 genome copies as determined using genomic DNA as template. Each RPA assay was specific to the corresponding infected stool samples screened.

Development of multiplexed RPA assays for STH

The multiplexed detection of N. americanus and An. Duodenale including the amplification of a B. subtilis internal amplification control (IAC).

LOD of multiplex assay. An. duodenale was detected down to 10 plasmid copies in the presence of IAC, suggesting that multiplexing the reaction and addition of IAC did not affect assay performance.

Similar approach was taken for A. lumbricoides and T. trichiura. Multiplexed detection was achieved with either Ascaris-positive (SS6) or T. trichiura-positive (SS43) stools (based on KK and PCR), and A. lumbricoides and T. trichiura were amplified only in either SS6 or SS43, respectively.

SUMMARY AND CONCLUSIONS

STH RPA assays were developed to detect A. lumbricoides, T. trichiura, An. duodenale, and N. americanus. Each assay is rapid, sensitive, and specific. The assays were combined to create two multiplexed reactions that include an IAC to confirm the integrity of test reagents. Their performance is similar to real-time PCR but only takes 20 minutes to complete. Under optimal conditions, the LOD for each assay was very low (10-20 copies) and each assay was demonstrated to detect its target from STH-infected stool.

FUTURE WORK

- Continue the development of multiplexed RPA assays for simultaneous detection of STH from stool.
- Develop an improved DNA extraction procedure for stool.
- Evaluate performance on clinical specimens from STH-endemic region.

REFERENCES


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