DEVELOPMENT OF MODIFIED ALKALINE LYSIS – MAGNETIC BEAD EXTRACTION OF DNA FOR MOLECULAR DIAGNOSIS OF SOIL-TRANSMITTED HELMINTHS FROM STOOL

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BACKGROUND

Soil-transmitted helminths (STH) are parasitic intestinal worms that are controlled with mass drug administration (MDA) in at-risk populations.

A recombinase polymerase amplification (RPA)-based nucleic acid amplification test is being developed to address the need for a more sensitive diagnostic for assessing the impact of MDA, to guide control program decisions (such as determining when to reduce or stop MDA), and for post-MDA surveillance.

For molecular diagnosis of STH infection, STH eggs in stool are the only recommended biomarker due to the biology of helminth infections. A stool-processing technique to effectively lyse STH eggs and extract amplifiable target DNA is necessary prior to any nucleic acid testing. A commercially available spin column–based stool DNA extraction kit, though effective, is comparatively expensive, requires centrifuge, and is not suitable for field use.

OBJECTIVE

To develop a rapid, field-deployable, non-instrumented, magnetic bead-based protocol to extract STH egg DNA in stool, using Ascaris suum as model species, for use as a companion tool to RPA-based diagnostics for STH.

MATERIALS AND METHODS

Sources of materials. Ascaris suum eggs and stool specimens were from Excelsior Sentinel, Inc. (Trumansburg, NY) and BioreclamationIVT (Baltimore, MD), respectively. Magnetic beads were from Amsbio LLC (Cambridge, MA). PowerFecal®, DNA isolation kit was purchased from MOBio (Carlsbad, CA). TwistAmp® exo kits for RPA reactions were from TwistDx (Cambridge, UK).

Optimization of lysis conditions and DNA capture steps. Different egg lysis conditions were tested—including alkaline lysis buffers (20 or 200 mM KOH ± 50% PEG 200), bead beating, heating, or a combination thereof using a factorial design of experiment—and the results were analyzed using Minibat 7. DNA-binding buffers (either potassium acetate or guanidinium-based), magnetic bead types (plain silica vs carboxylated, and 1.2 μm vs 3 μm in size), and wash buffers (with varying concentrations of alcohol) were evaluated.

Egg-spiking experiments. Stools (200mg) were spiked with Ascaris eggs at different levels (EPG) corresponding to light and moderate infection intensities, resuspended in modified alkaline lysis (MAL) buffer, bead-bashed, and heated. Lysates were either tested directly (MAL) or subjected to DNA-capture steps using silica magnetic beads (MAL–MB), and benchmarked against PowerFecal® (MOBIO) and QIAGEN stool DNA extraction protocol (Verweij, 2007).

Molecular analyses. Eluted DNA was subjected to either Ascaris-RPA assay (Cantera et al, unpub) using TwistAmp® (TwistDx) or real-time PCR assays (Verweij 2007; Mejia 2013) using Mx3005P qPCR system (Agilent Technologies).

Modified alkaline lysis – magnetic bead (MAL–MB) general workflow. MAL–MB offers the following advantages versus spin column method: (1) simpler procedure with no requirement for centrifuge, (2) involves fewer steps as it eliminates moving samples in and out of centrifuge and decreasing processing time, and (3) has high throughput capability (using multi-well magnetic plates or multipronged extractors).

RESULTS

Effective lysis of Ascaris eggs was achieved using modified alkaline lysis (MAL) buffer, bead-bashing, and heat

MAL–MB allowed detection of target DNA in stools with low levels of Ascaris eggs, with performance similar to MOBIO DNA extraction kit

MAL–MB yielded more DNA but with lesser purity than other spin column–based DNA extraction protocol

SUMMARY AND CONCLUSION

A magnetic bead-based DNA capture protocol (MAL–MB) was developed to effectively lyse eggs and extract total DNA from stools. The MAL–MB performed similarly as the MOBIO DNA extraction kit and provided amplifiable DNA from stools with low levels of Ascaris eggs corresponding to low density of infection for RPA. This centrifuge-free technique for stool DNA extraction would be useful as a companion tool for RPA-based molecular diagnosis of STH, designed for use in field surveillance of STH infections.

FUTURE PERSPECTIVE

- Improve DNA capture and wash steps for more consistent results.
- Evaluate MAL–MB performance on Trichuris-spiked stools as well as clinical samples, and benchmark against standard DNA extraction kit.
- Identify and validate disposable kit components for field use.

REFERENCES


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