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 lyze 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.

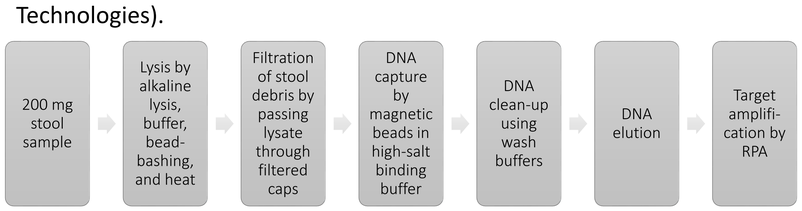
MATERIALS AND METHODS

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 ± 60% PEG 200), bead beating, heating, or a combination thereof using a factorial design of experiment—and the results were analyzed using Minitab7. DNA-binding buffers (either potassium acetate or guanidinium-based), magnetic bead types (plain silica vs carboxylated, and 1.2 µm vs 3.0 µm 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).

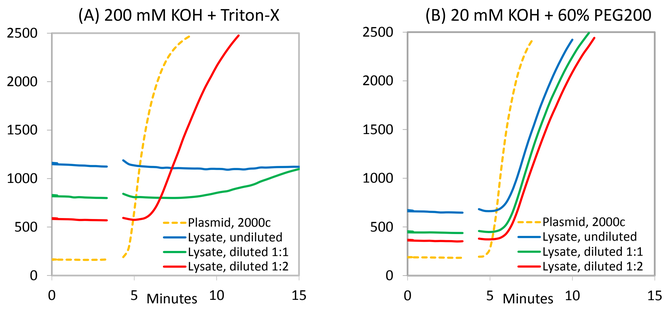
Molecular analyses. Eluted DNA was subjected to either Ascaris-RPA assay (Cantera et al, unpub) using Twista® (TwistDx) or real-time PCR assays (Verweij 2007; Mejia 2013) using Mx3005P qPCR system (Agilent Technologies).



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 decrease 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



- Eggs (15000 EPG) were lyzed effectively using a combination of either lysis buffer (A) or (B), bead-bashing, and heat. Non-amplification of target in undiluted and diluted (1:1) lysate in (A) could be due to high KOH concentration that may have affected RPA reactions.

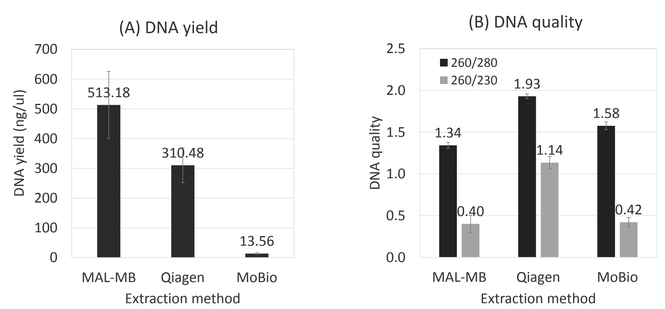
MAL–MB allowed detection of target DNA in stools with low levels of *Ascaris* eggs, with performance similar to MOBIO DNA extraction kit

Infection intensity	No. of spiked *Ascaris* eggs (EPG)	No. of RPA-positive replicates				
		MAL, diluted 1:4	MAL-MB (200, 1)*	MAL-MB (500, 2)*	MOBIO	QIAGEN
Moderate	15,000	1/3	4/4	10/10	6/6	10/10
	5000	1/3	4/4	10/10	6/6	15/16
Light	2500	1/3	4/4	-	-	-
	500	0/3	5/5	-	-	-
	250	0/3	5/5	14/14	6/6	6/20
	125	0/3	3/5	12/14	6/6	2/20
	50	0/3	2/5	13/14	3/6	4/20
Unspiked	0	0/3	0/5	0/14	0/6	0/10

(*) Numbers in brackets are lysate volume for DNA capture and input DNA for RPA, respectively. Dash (-), not determined.

- MAL produced inconsistent amplification, with or without dilution of lysate. Addition of DNA capture step (MAL–MB) allowed detection of target DNA from stools with low levels of eggs. Increasing the lysate volume for extraction and input DNA for RPA in MAL–MB (500, 2) allowed detection of 50-125 EPG, although not significantly different than MAL–MB (200, 1) ($P = 0.53$, Chi^2-test at 95% CI).
- MAL–MB showed no significant difference with MOBIO (T-test, $P = 0.062$), while MAL–MB performed better than QIAGEN (T-test, $P = <0.001$) in terms of the number of RPA-positives produced.

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



- Total extracted DNA from egg-spiked stools were analyzed by Nanodrop-1000 for DNA yield and purity. Values shown are averages from 15 replicate samples. MAL–MB gave the highest DNA yield but with the lowest purity. MOBIO gave the lowest yield but better purity than MAL–MB. QIAGEN gave a better yield and best quality DNA.

RESULTS continued

Target detection in MAL–MB extracted DNA from different stool lots containing lows levels of *Ascaris* eggs

Stool lot#	EPG	DNA yield (ng/µL) ± SD		Mean DNA yield	260/280	260/230	RPA results
310	250	191.5	169.8	151.7	1.4	0.6	4/6
	125	153.9	127.7				3/6
	50	109.7	25.6				0/6
309	250	521.7	382.6	458.0	1.4	0.5	1/6
	125	474.7	25.6				3/6
	50	377.6	84.3				1/6
317	250	582.5	237.8	414.5	1.2	0.4	6/6
	125	460.9	172.4				4/6
	50	200.0	75.4				1/6
192	250	539.0	229.9	631.3	1.3	0.4	5/6
	125	901.0	126.6				3/6
	50	453.8	334.3				1/6
313	250	103.3	83.8	119.7	1.3	0.5	2/6
	125	107.8	59.6				0/6
	50	148.1	90.4				3/6

- There was a large variance in mean DNA yield in replicates of the same stool lot, probably due to variation in stool composition that may have affected the DNA capture step.

SUMMARY AND CONCLUSION

A magnetic bead-based DNA capture protocol (MAL–MB) was developed to effectively lyze 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 intensity 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-based 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 kitting.

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