Comparison of five methods for recovery of *Mycobacterium tuberculosis* DNA from stool samples

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PROTOCOLS

QIAamp DNA Stool Mini Kit

This kit performs the DNA extraction based in silica affinity, which retain DNA for purification

Fig 1. QiAamp DNA Stool Mini Kit.

Microsens TB-Beads

Kit includes Magnetic microbeads which bind specifically to mycobacteria and retain these cells by load affinity.

Fig 2. Microsens TB-Beads Kit.

MoBio PowerFecal DNA Isolation Kit

Kit designed for purification of both microbial and host genomic DNA from stool and feces, based on membranes silica which retain strands of DNA

Fig 3. MoBio PowerFecal DNA Isolation Kit.

Akonni Biosystems automated TruTip Kit

Automated method which uses columns with membranes silica contained in Tips which retain DNA strands for purification.

Fig 4. Akonni Biosystems automated TruTip Kit.

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OBJECTIVE

As part of a larger study evaluating new tools for diagnosis of tuberculosis in children, we set out to select an optimal DNA extraction method for *Mycobacterium tuberculosis* from stool samples.

INTRODUCTION

Laboratory diagnosis of pulmonary tuberculosis is sputum based but in patients who cannot produce sputum (i.e., children) invasive procedures, such as gastric aspirates, may be used to detect or diagnose TB.

Stool and other non-invasive samples could be an alternative for diagnosis because is possible to find TB cells, however, the presence of specimen inhibitors is a challenge for molecular testing.

In this work, we compared five different DNA extraction kits to determine which method maximizes the DNA recovery while minimizing the presence of PCR inhibitors. All this will be assessed by real time PCR.

1. QiAamp DNA Stool Mini Kit.
2. QiAamp DNA Stool Mini Kit with Microsens TB-Beads.
3. MoBio PowerFecal DNA Isolation Kit.
4. Akonni Biosystems automated TruTip Kit.
5. Akonni TruTip with Microsens TB-Beads.

MATERIALS AND METHODS

Stool samples from five healthy Peruvian adult volunteers, divided into two aliquots each.

Samples were inoculated with mycobacterial suspension (0.5 McFarland) solutions of H37Ra *Mtb*, declumped with beads in Nuclease free water.

DNA Extraction

Controls for each method:
Positive: *Mtb* cells
Negative: Only Stool
System: Only Water

- QIAamp DNA Stool mini kit (0.3 g.)
- QiAamp DNA Stool Mini Kit with Microsens TB-Beads (5.0 g.)
- MoBio PowerFecal DNA Isolation Kit (0.3 g.)
- Akonni Biosystems automated TruTip Kit (5.0 g.)
- Akonni TruTip with Microsens TB-Beads (5.0 g.)

Real Time PCR → IS6110 targeted using hydrolisis probes, in a LightCycler 480 System (Roche)

Detection Format	Mono Color Hydrolisis probe: FAM (465-510)				
Program Name	Cycles	Analysis Mode	Temperature	Time	Acquisition
Denaturation	1	None	95ºC	10 min.	
PCR	45	Quantification	95ºC	15 sec.	
			60ºC	60 sec.	Single

Protocol

Analysis: Absolute quantification, using the second derivative maximum.

Inhibition test: The eluates from the high concentration samples were diluted ten-fold and amplified by real-time PCR alongside the non-diluted samples.

Percent inhibition was calculated by comparing the concentration of the diluted to the undiluted sample

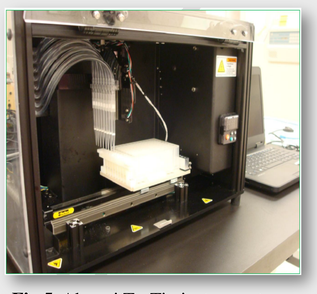


Fig 5. Akonni TruTip instrument

Fig 6. LightCycler 480 instrument

RESULTS

Table 1. Total number of extractions performed

EXTRACTION METHOD	Number of samples		# of controls	TOTAL
	Low Mtb concentration	High Mtb concentration		
Qiagen	5	5	3	13
Qiagen+Beads	5	5	3	13
TruTip	5	5	6	16
TruTip+Beads	5	5	6	16
PowerFecal	5	5	3	13
TOTAL	25	25	21	71

Fig 7. Amplification curves obtained from the LightCycler 480

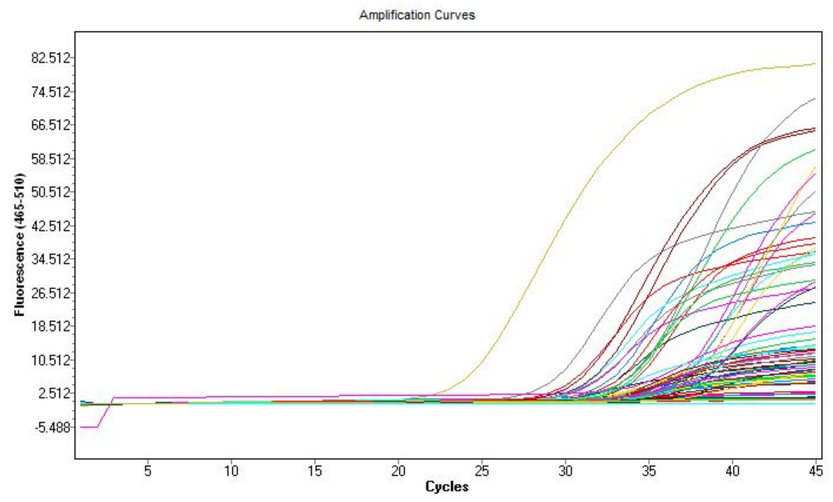
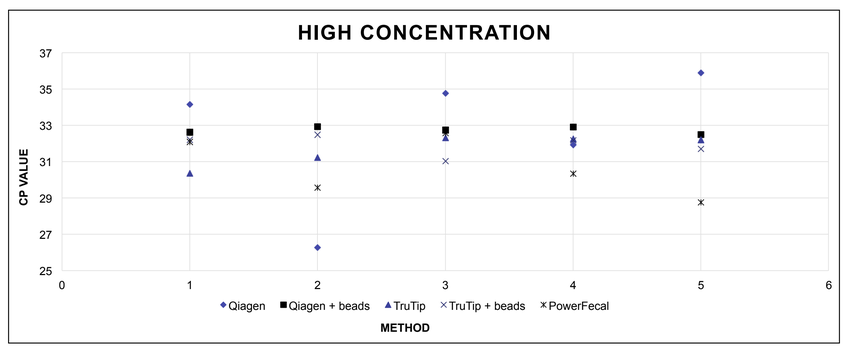


Fig 8. Scatter plot for Cp values obtained from High (A) and Low (B) concentration samples.

A)



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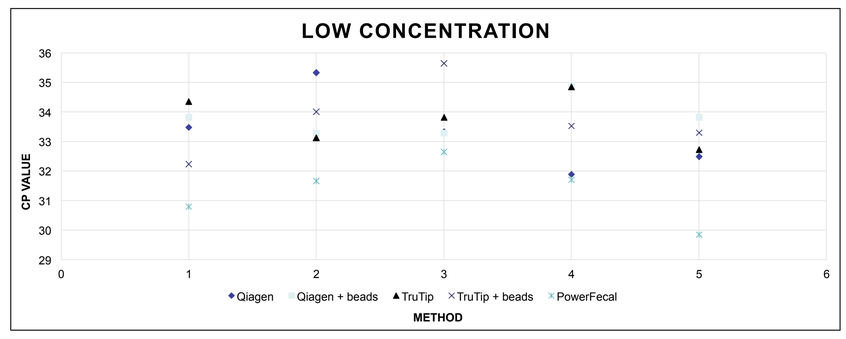

Table 2. Summary Real Time PCR results: Mean Cp values and DNA yield

Extraction method	Cp Value		Concentration (pg/ul)		Inhibition (%)
	Low Conc.	High Conc.	Low Conc.	High Conc.	
Qiagen	33.3	32.6	0.013	0.015	No amp.
Qiagen + beads	33.8	32.7	0.008	0.015	54
TruTip	**33.8**	**31.7**	**0.009**	**0.032**	**52**
TruTip + beads	33.7	31.9	0.010	0.025	31
PowerFecal	**31.3**	**30.7**	**0.041**	**0.073**	**52**

No negative or system controls showed amplification on the Real Time PCR.
All positive controls showed amplification on the Real Time PCR, with an average Cp Value of 32.2.

DISCUSSION AND CONCLUSIONS

Results show that the MoBio PowerFecal DNA Isolation Kit (mean Cp values 30.7 [high concentration] and 31.3 [low concentration]), followed by Akonni TruTip (Cp value 31.7 [high concentration] and 33.8 [low concentration]), are the most optimal methods for the recovery of DNA of *Mycobacterium tuberculosis* from stool samples inoculated with mycobacterial suspensions.

These methods should be tested with clinical samples to determine real levels of inhibition and which method would be the most effective to enhance the diagnosis of *Mycobacterium tuberculosis* in children.