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Is Supplementary Bead Beating for DNA Extraction from Nematode Eggs by Use of the NucliSENS easyMag Protocol Necessary?

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Recent studies have demonstrated the effectiveness and applicability of PCR-based methods for the detection of intestinal helminths in stool samples (1–5); however, successful DNA extraction is essential. We compared DNA extractions using NucliSENS easyMag, with and without a preceding step of supplementary bead beating, from eggs of parasitic nematodes by spiking equal portions of *Dientamoeba fragilis*-positive stool samples with eggs of *Ascaris suum* and *Trichuris trichiura*. *A. suum* eggs isolated from pig feces were washed three times in phosphate-buffered saline (PBS), and 0, 1, 3, 5, and 20 eggs were spiked into triplicate samples of 100 mg feces (Table 1). To each sample, we either added no beads or 150 to 200 mg of one of three different types of beads: 0.5-mm glass beads (CoBio, Copenhagen, Denmark), 0.15-mm garnet beads (CoBio, Copenhagen, Denmark), or 0.1-mm zirconium beads (Techtum Lab AB, Umeå, Sweden). The samples were then either vortexed on a Vortex-Genie 2 for 10 min at 2,850 rpm or shaken by bead beating (BB) for 30 s at 7,000 oscillations on a MagNA Lyser. Subsequent extraction using NucliSENS easyMag (bioMérieux, Denmark) was performed according to the manufacturer's recommendations (protocol B, 60 μl silica). Samples were analyzed for *D. fragilis*, *A. suum*, and *T. trichiura* by quantitative PCR (qPCR).

Next, we tested a clinical fecal sample positive for *T. trichiura*. Using PBS, we prepared a triplicate dilution series of the clinical sample (Table 2). To each sample, we added either no beads or zirconium beads, since this type of bead gave the best results in the first experiment. Samples were either vortexed or subjected to bead beating before DNA extraction using NucliSENS easyMag. All samples were analyzed for *T. trichiura* by qPCR in duplicate reactions.

Overall, no loss of *D. fragilis* DNA was detected, indicating the absence of DNA degradation (data not shown). The standard NucliSENS easyMag protocol was generally comparable to the bead beating modification regardless of bead type and mixing method, the only exception being zirconium beads combined with bead beating (Z-BB), in which case the diagnostic sensitivity was 100% (24/24) and the lower limit of detection was 10 eggs per gram of feces (Table 1). However, Z-BB did not result in more PCR-positive samples in any of the *T. trichiura* setups. The detection limit for the spiked *T. trichiura* samples was >20 eggs/100 mg (fecal egg count [FEC] = 200), i.e., higher than for the clinical sample (FEC, ~76 eggs/gram [100-fold dilution]), indicating non-*T. trichiura* egg DNA present in the clinical sample.

Analysis of weighted changes in mean C_T values (Table 3) showed that only for *A. suum* did the use of Z-BB result in a significant increase in sensitivity compared to that of vortexing with zirconium beads (Z-V), the weighted mean ΔC_T value of 1.65 being higher than the observed weighted mean standard deviation of 1.19.

Clogging of easyMag tips happened for more than two-thirds of samples processed with glass beads (data not shown).

Conclusively, bead beating with zirconium beads (Z-BB) prior to the NucliSENS easyMag DNA extraction from fecal samples

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TABLE 1 Experimental setup and qPCR results of the spiking DNA extraction experiments on human fecal samples[a]

Nematode species	Extraction conditions		Mean C_T value (SD) for samples spiked with:					No. of positive PCRs out of 6 possible for samples spiked with:				
	Mixing method	Beads[b]	0 eggs	1 egg	3 eggs	5 eggs	20 eggs	0 eggs	1 egg	3 eggs	5 eggs	20 eggs
A. suum	Vortexing	None	—	—	39.82	38.85 (1.85)	38.04 (3.03)	—	—	1	4	4
	Vortexing	Zirconium	NA	—	40.69 (0.94)	39.91 (0.55)	39.53 (1.69)	NA	—	2	3	6
	Vortexing	Garnet	NA	—	36.81 (1.53)	41.32 (1.13)	40.74 (0.87)	NA	—	6	3	4
	BB	Zirconium	NA	40.40 (1.08)	37.78 (2.21)	37.75 (1.30)	39.05 (0.86)	NA	6	6	6	6
	BB	Garnet	NA	41.18	39.70 (0.61)	38.24 (1.76)	39.97 (1.51)	NA	1	4	3	3
T. trichiura	Vortexing	None	—	—	41.16	40.36	39.58 (0.02)	—	—	1	1	2
	BB	Zirconium	—	—	—	39.26 (1.63)	37.64 (0.56)	—	—	—	4	2

[a] Artificial spiking of nematode-negative, *Dientamoeba fragilis*-positive samples with eggs of *A. suum* and *T. trichiura*. Samples are in triplicate and were analyzed in duplicate reactions; mean C_T values are shown. NA, not applicable; BB, bead beating; —, sample negative by qPCR.
[b] Glass beads were not included in the table (see the text for details).

TABLE 2 Experimental setup and qPCR results of the dilution DNA extraction experiments on human fecal samples[a]

Extraction conditions		Mean C_T value (SD) at indicated dilution					No. of positive PCRs of 6 possible at indicated dilution				
Mixing method	Beads[b]	1,000 (0.76 egg/100 mg)	500 (1.52 eggs/100 mg)	100 (7.6 eggs/100 mg)	10 (76 eggs/100 mg)	1 (760 eggs/100 mg)	1,000 (0.76 egg/100 mg)	500 (1.52 eggs/100 mg)	100 (7.6 eggs/100 mg)	10 (76 eggs/100 mg)	1 (760 eggs/100 mg)
Vortexing	None	43.27	38.50	37.39	36.41	34.34	2	3	5	6	6
BB	Zirconium	44.98	39.51	37.70	35.69	33.90	1	4	6	6	6

[a] Dilution of a *Trichuris trichiura*-positive, *D. fragilis*-negative clinical sample (the original sample had a fecal egg count of 7,600 eggs/g). Samples are in triplicate and were analyzed in duplicate reactions; mean C_T values are shown.
[b] Glass beads were not included in the table (see the text for details).

TABLE 3 Display of mean ΔC_T values obtained by qPCR for the different modifications

Parameter	Organism	Mixing method	Beads	Modifications[a]				
Spiking expt	*A. suum*	Vortexing (V)	Zirconium (Z)	Z-V		Z-V		
	A. suum	Vortexing (V)	Garnet (G)	G-V			G-V	
	A. suum	Bead beating (BB)	Zirconium (Z)		Z-BB	Z-BB		
	A. suum	Bead beating (BB)	Garnet (G)		G-BB		G-BB	
	T. trichiura	Vortexing (V)	None (E)					E-V
	T. trichiura	Bead beating (BB)	Zirconium (Z)					Z-BB
Mean ΔC_T value[b]				1.26	−4.12	5.55	0.97	3.05
Weighted mean ΔC_T value[c]				0.78	−1.15	1.65	−0.28	1.12
Weighted mean SD[d]				1.15	1.36	1.19	1.31	—[e]

[a] See the text for details of the different modifications. In each of the five columns, two modifications corresponding to boxes shaded in gray are compared. A positive value means that the latter method (top-bottom orientation) has a lower C_T value than the former method and vice versa.
[b] Each mean ΔC_T value is calculated for each modification for samples containing 3 to 20 eggs for *A. suum* and 5 to 20 eggs for *T. trichiura*.
[c] Each mean ΔC_T value is weighted against the number of positive qPCR results in Table 1; for instance, for *T. trichiura*, $(40.36 \cdot 1 + 39.58 \cdot 2)/(1 + 2) - (39.26 \cdot 4 + 37.64 \cdot 2)/(4 + 2) = 1.12$.
[d] Each weighted mean standard deviation (SD) is calculated based on the SD for 5 to 20 eggs samples for *A. suum* (data not shown) and weighted as described above.
[e] —, only the experiment with 20 eggs resulted in >1 positive qPCR test, and so a weighted SD could not be calculated.

results in no loss in the DNA yield of *D. fragilis* and a slight increase in DNA yield from eggs of *Ascaris* but not *Trichuris*.

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