**Improved diagnosis of *Trichuris trichiura* by using a bead-beating procedure on ethanol preserved stool samples prior to DNA isolation and the performance of multiplex real-time PCR for intestinal parasites.**

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**Supplementary Information**

**Table S1.** The comparison of cycle threshold (Ct) -values for different bead types tested in bead-beating procedure for *T. trichiura* detection

|  |  |  |
| --- | --- | --- |
| Sample ID | Egg count\* | Real-time PCR (Ct-value) |
| C\_PCR | Type of beads used in B\_PCR\*\* |
| Stainless Steel | Zirconium Oxide | Matrix A | Garnet | Matrix Y |
| A1284 | 61 | 34.18 | 27.13 | 24.69 | 25.14 | 26.58 | 26.40 |
| A1311 | 42 | 34.33 | 27.15 | 26.91 | 27.13 | 26.00 | 0 |
| D537 | 2 | 0 | 0 | 37.01 | 0 | 33.97 | 0 |
| D680 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| H3404 | 162 | 37.06 | 32.48 | 34.98 | 31.52 | 29.15 | 35.03 |
| H4022 | 374 | 37.75 | 32.87 | 28.06 | 26.32 | 27.72 | 27.55 |

C\_PCR=PCR resulted from directly frozen sample; B\_PCR=PCR resulted from bead-beating supplemented on frozen sample.

\* A single slide microscopy examination performed using formol-ether concentration procedure

\*\* The sizes of the beads are the following: Stainless steel [nextadvance, NY] is 0.5 mm; Zirconium Oxide [nextadvance, NY] is 0.5 mm; Matrix A is a 0.7 mm Garnet Lysing matrix [MP Biomedicals]; Garnet is 0.8 mm bead [Mobio US, SanBio Netherlands]; Matrix Y are 0.5 mm Yttria-Stabilized Zirconium Oxide beads [MP Biomedicals]. The volume of bead used per sample was 1.072 cm3, this volume corresponds to 0.50 gram, 0.30 gram, 0.15 gram, 0.25 gram and 0.30 gram of beads which are, respectively, steel, zirconium oxide, matrix A, garnet and Matrix Y.

**Table S2.** Oligonucleotide primers and detection probes for real-time PCR for the simultaneous detection of intestinal helminth and protozoa

|  |  |  |  |
| --- | --- | --- | --- |
| Target organism | Oligo name | Oligonucleotide sequences | Reference |
| *Schistosoma* sp. | Ssp\_ITS\_48F | 5’- GGTCTAGATGACTTGATYGAGATGCT -3’ |  (Obeng *et al.*, 2008) |
| Ssp\_ITS\_124R | 5’- TCCCGAGCGYGTATAATGTCATTA -3’ |
| Ssp\_ITS\_78T\_FAM | FAM-5’- TGGGTTGTGCTCGAGTCGTGGC -3’-BHQ1 |
| *Trichuris trichiura* | Tt\_283F | 5’- TTGAAACGACTTGCTCATCAACTT -3’ | (Liu *et al.*, 2013) |
| Tt\_358R | 5’- CTGATTCTCCGTTAACCGTTGTC -3’ |
| Tt\_308T\_YY | Yakima Yellow-5’- CGATGGTACGCTACGTGCTTACCATGG -3’-BHQ1 |
| *Ancylostoma* sp. | Ad\_125F | 5’- GAATGACAGCAAACTGCTTGTTG -3’ | (Hamid *et al.*, 2011; Verweij *et al.*, 2009; Wiria *et al.*, 2010) |
| Ad\_195R | 5’- ATACTAGCCACTGCCGAAACGT -3’ |
| Ad\_155\_XS\_TR | Texas red-5’- ATCGTTTACCGACTTTAG -3’BHQ2 |
| *Necator americanus* | Na\_58F | 5’- CTGGTTTGTCGAACGGTACTTGC -3’ |
| Na\_158R | 5’- ATAACAGCGTGCACATGTTGC -3’ |
| Na\_81T\_XS\_FAM | FAM-5’- CTGTACTACGCATTGTATAC -3’-BHQ1 |
| *Ascaris lumbricoides* | Alum\_96F | 5’- GTAATAGCAGTCGGCGGTTTCTT -3’ |
| Alum\_183R | 5’- GCCCAACATGCCACCTATTC -3’ |
| Alum\_124T\_YY | Yakima Yellow-5’- TTGGCGGACAATTGCATGCGAT -3’-BHQ1 |
| *Strongyloides stercoralis* | Stro 18S-1530F | 5’- GAATTCCAAGTAAACGTAAGTCATTAGC -3’ |
| Stro 18S-1630R | 5’- TGCCTCTGCATATTGCTCAGTTC -3’ |
| Stro 18S-1586T | Quasar705-5’- ACACACCGGCCGTCGCTGC -3’-BHQ2 |
| *Entamoeba histolytica* | Ehd\_F | 5’- ATTGTCGTGGCATCCTAACTCA -3’ | (Verweij *et al.*, 2003a) |
| Ehd\_R | 5’- GCGGACGGCTCATTATAACA -3’ |
| Eh\_18S\_XS\_YY | Yakima Yellow-5’- TCATTGAATGAATTGGCCATTT -3’-BHQ1 |
| *Dientamoeba fragilis* | Df\_124F | 5’- CAACGGATGTCTTGGCTCTTTA -3’ | (Verweij *et al.*, 2007) |
| Df\_221R | 5’- TGCATTCAAAGATCGAACTTATCAC -3’ |
| Df\_172\_XS\_Quasar705 | Yakima Yellow-5’- CAATTCTAGCCGCTTAT -3’-BHQ1 |
| *Giardia lamblia* | Giardia\_18S\_99F | 5’- GACGGCTCAGGACAACGGTT -3’ | (Verweij *et al.*, 2004; Verweij *et al.*, 2003b) |
| Giardia\_18S\_125R | 5’- TTGCCAGCGGTGTCCG -3’ |
| Giardia\_18S\_FAM | FAM 5’- CCCGCGGCGGTCCCTGCTAG -3’-BHQ1 |
| *Cryptosporidium* spp. | Cr\_spp\_JVAF  | 5’- ATG ACG GGT AAC GGG GAAT -3’ | (Jothikumar *et al.*, 2008) |
| Cr\_spp\_JVAR  | 5’- CCA ATT ACA AAA CCA AAA AGT CC -3’ |
| Cr\_spp\_JVAP18S\_TR | Texas Red 5’- CGC GCC TGC TGC CTT CCT TAG ATG -3’-BHQ2  |
| Phocin Herpes Virus (PhHV) | PhHV\_267s | 5’- GGGCGAATCACAGATTGAAT|C -3’ | (Niesters, 2002) |
| PhHV\_337as | 5’- GCGGTTCCAAACGTACCAA -3’ |
| PhHV\_305tq\_Cy5 | Cy5-5’- TTTTTATGTGTCCGCCACCATCTGGATC -3’-BHQ2 |

**Table S3.** Mixtures composition in three real-time PCR panels used for intestinal parasites detection

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| --- |
| Panel I: ST |
| Reagents: | Concentration: | 1 Sample: |
| H20 |  | 2,025 |
| MgCl2 | 25 mM | 3,50 |
| BSA | 5 mg/ml | 0,50 |
| Primer Ssp -F | 25 µM | 0,20 |
| Primer Ssp -R | 25 µM | 0,20 |
| Probe Ssp -FAM | 10 µM | 0,125 |
| Primer Tt -F | 25 µM | 0,20 |
| Primer Tt -R | 25 µM | 0,20 |
| Probe Tt -YY | 10 µM | 0,125 |
| Primer PHHV -S | 25 µM | 0,15 |
| Primer PHHV -AS | 25 µM | 0,15 |
| Probe PHHV - Cy5 | 10 µM | 0,125 |
| HotStar Taq Master Mix |  | 12,50 |
| Total |  | 20,00 |
| Add 5 µl DNA to the mix |  |  |

|  |
| --- |
| Panel II: ANAS |
| Reagents : | Concentration: | 1 Sample: |
| H20 |   | 2,025 |
| MgCl2 | 25 mM | 3,50 |
| BSA | 5 mg/ml | 0,50 |
| Primer Ad -F  | 25 µM | 0,20 |
| Primer Ad -R  | 25 µM | 0,20 |
| Probe Ad - TR  | 10 µM | 0,25 |
| Primer Na -F  | 25 µM | 0,20 |
| Primer Na -R | 25 µM | 0,20 |
| Probe Na - FAM | 10 µM | 0,125 |
| Primer Alum -F | 10 µM | 0,20 |
| Primer Alum -R | 10 µM | 0,20 |
| Probe Alum - YY | 10 µM | 0,125 |
| Primer Stro -F | 25 µM | 0,10 |
| Primer Stro -R | 25 µM | 0,10 |
| Probe Stro -Quasar 705 | 10 µM | 0,125 |
| Primer PHHV -S | 25 µM | 0,15 |
| Primer PHHV -AS | 25 µM | 0,15 |
| Probe PHHV - Cy5 | 10 µM | 0,125 |
| HotStar Taq Master Mix |  | 12,50 |
| Total |  | 20,00 |
| Add 5 µl DNA to the mix |  |  |

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| --- |
| Panel III: HDGC |
| Reagents: | Concentration: | 1 Sample: |
| H20 |  | 1,61 |
| MgCl2 | 25 mM | 3,50 |
| BSA | 5 mg/ml | 0,50 |
| Primer Ehd -F | 25 µM | 0,04 |
| Primer Ehd -R | 25 µM | 0,04 |
| Probe Eh - YY | 10 µM | 0,125 |
| Primer Df -F | 25 µM | 0,20 |
| Primer Df -R | 25 µM | 0,20 |
| Probe Df -Quasar705 | 10 µM | 0,25 |
| Primer Giardia -F | 25 µM | 0,06 |
| Primer Giardia -R | 25 µM | 0,06 |
| Probe Giardia -FAM  | 10 µM | 0,125 |
| Primer Cr spp -F | 25 µM | 0,06 |
| Primer Cr spp -R | 25 µM | 0,06 |
| Probe Cr spp -TR | 10 µM | 0,25 |
| Primer PHHV - S | 25 µM | 0,15 |
| Primer PHHV -AS | 25 µM | 0,15 |
| Probe PHHV - Cy5 | 10 µM | 0,125 |
| HotStar Taq Master Mix |  | 12,50 |
| Total |  | 20,00 |
| Add 5 µl DNA to the mix |  |  |