|  |  |  |  |  |  |  |  |
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| **PCR conditions and primers** |  |  | | | | |  |
| **Target genes** | **Primers** | **Cycling conditions** | | | | | **References** |
| ***Cryptosporidium: s*mall subunit (SSU) rRNA** | | | | | | |  |
| First amplification,  Amplicon size: 1325 bp | SSU F1: (5’ TTCTAGAGCTAATACATGCG-3’)  SSU R1: (5’-GACGAGCCTCTGCACAACATC-3’) | | 95°C, 3 min | | | | Jiang et al., 2005 |
| 94°C, 45 sec | |  | |
| 55°C, 45 sec | | 35x | |  |
| 72 °C, 60 sec | |  | |  |
| 72 °C, 7 min | | | |
| Second amplification  Amplicon size: 840 bp | SSU F2: (5’ GGAAGGGTTGTATTTATTAGATAAAG-3’)  SSU R2: (5’-CTCATAAGGTGCTGAAGGAGTA-3’) | | 95°C, 3 min | | | | Jiang et al., 2005 |
| 94°C, 45 sec |  | | |
| 55 °C, 45 sec | 35x | | |
| 72 °C, 60 sec |  | | |
| 72 °C, 7 min |  | | |
| ***Cryptosporidium ubiquitum: 60-kDa glycoprotein*** | | | | | | | Li et al., 2014  Li et al., 2014 |
| First amplification,  Amplicon size: 1044 bp | F: (5’- TTTACCCACACATCTGTAGCGTCG -3’) | | 95°C, 3 min | | | |
| R: (5’- ACGGACGGAATGATGTATCTGA-3’) | | 94°C, 45 sec | | |  |
|  | | 58°C, 45 sec | | | 35x |
|  | | 72 °C,60 sec | | |  |
|  | | 72 °C, 7 min | | | |
| Second amplification  Amplicon size: 948 bp | F: (5’- ATAGGTGATAATTAGTCAGTCTTTAAT-3’) | | 95°C, 3 min | | | |
| R: (5’- TCCAAAAGCGGCTGAGTCAGCATC-3’) | | 94°C, 45 sec | | |  |
|  | | 55°C, 45 sec | | | 35x |
|  | | 72 °C, 60 sec | | |  |
|  | | 72 °C, 7 min | | | |
| ***Giardia: s*mall subunit (SSU) rRNA** | | | | | | | Read et al., 2002 |
| First amplification  Amplicon size: 292 bp | F: RH11(5’-CATCCGGTCGATCCTGCC-3’) | | 95°C, 3 min | | | |
| R: RH4 (5’-AGTCGAACCCTGATTCTCCGCCAGG-3’) | | 96°C, 30 sec | | |  |
|  | | 59°C, 40 sec | | | 40x |
|  | | 72 °C, 40 sec | | |  |
|  | | 72 °C, 7 min | | | |
| Second amplification  Amplicon size: 175 bp | F: GiarF (5’-GACGCTCTCCCCAAGGAC-3’) | | 95°C, 3 min | | | |
| R: GiarR (5’-CTGCGTCACGCTGCTCG-3’) | | 96°C, 30 sec | | |  |
|  | | 55°C, 40 sec | | | 40x |
|  | | 72 °C, 30 sec | | |  |
|  | | 72 °C, 7 min | | | |
| ***Giardia*: glutamate dehydrogenase (GDH)** | | | | | | | Cacciò et al., 2008 |
| First amplification  Amplicon size: 755 bp | F: GDH1 (5’-TTCCGTRTYCAGTACAACTC-3’) | | 95°C, 3 min | | | |
| R: GDH2 (5’-ACCTCGTTCTGRGTGGCGCA-3’) | | 94°C, 30 sec | | |  |
|  | | 50°C, 30 sec | | | 35x |
|  | | 72 °C, 60 sec | | |  |
|  | | 72 °C, 7 min | | | |
| Second amplification  Amplicon size: 530 bp | F: GDH3 (5’-ATGACYGAGCTYCAGAGGCACGT-3’) | | 95°C, 3 min | | | |
| R: GDH4 (5’- GTGGCGCARGGCATGATGCA -3’) | | 94°C, 45 sec | | |  |
|  | | 54°C, 45 sec | | | 40x |
|  | | 72 °C, 45 sec | | |  |
|  | | 72 °C, 7 min | | | |
| ***Giardia*: beta giardin (BG)** | | | | | | | Lalle et al., 2005 |
| First amplification  Amplicon size: 753 bp | F: G7(5’-AAGCCCGACGACCTCACCCGCAGTGC-3’) | | 95°C, 3 min | | | |
| R: G759 (5’-GAGGCCGCCCTGGATCTTCGAGACGAC-3’) | | 94°C, 30 sec | | |  |
|  | | 60°C, 30 sec | | | 35x |
|  | | 72 °C, 60 sec | | |  |
|  | | 72 °C, 10 min | | | |
| Second amplification  Amplicon size: 511 bp | F: (5’- GAACGAGATCGAGGTCCG-3’) | | 95°C, 3 min | | | |
| R: (5’- CTCGACGAGCTTCGTGTT-3’) | | 95°C, 30 sec | | |  |
|  | | 53°C, 30 sec | | | 40x |
|  | | 72 °C, 60 sec | | |  |
|  | | 72 °C, 10 min | | | |
| ***Giardia:* Triosephosphate isomerase (TPI)** | | | | | | | Sulaiman et al., 2003 |
| First amplification  Amplicon size: 605 bp | F: (5’-AAATYATGCCTGCTCGTCG-3’)  R: (5’-CAAACCTTYTCCGCAAACC-3’) | | 95°C, 3 min | | | |
| 94°C, 45 sec | | |  |
| 50°C, 45 sec | | | 35x |
| 72 °C, 60 sec | | |  |
| 72 °C, 10 min | | | |
| Second amplification,  Amplicon size: 530 bp | F: (5’- CCCTTCATCGGIGGTAACTT -3’)  R: (5’- GTGGCCACCACICCCGTGCC-3’) | | 95°C, 3 min | | | | Sulaiman et al., 2003 |
| 94°C, 45 sec | | |  |
| 50°C, 45 sec | | | 35x |
| 72 °C, 60 sec | | |  |
| 72 °C, 10 min | | | |  |
| ***Giardia***: **NIMA-related kinase (**NEK) 15411 | | | | | | |  |
| First amplification  Amplicon size: 902 bp | F: (5’- TCTCCTCAACGACCCCTCAATC -3’)  R: (5’- CCGAGTCTACCTCACCACACTCAC-3’) | | 95°C, 3 min | | | | Ankarklev et al., 2018 |
| 95°C, 20 sec | |  | |
| 55°C, 30 sec | | 35x | |  |
| 72 °C, 45 sec | |  | |  |
| 72 °C, 7 min | | | |
| Second amplification  Amplicon size: 700 bp | F: (5’- GATGGCAATCGGCTTCTCC -3’)  R: (5’- CCGAGTCTACCTCACCACACTCAC-3’) | | 95°C, 3 min | | | | Ankarklev et al., 2018 |
| 95°C, 20 sec |  | | |
| 55°C, 30 sec | 35x | | |
| 72 °C, 45 sec |  | | |
| 72 °C, 7 min | | | |
| ***Giardia:* DNA repair and recombination protein RHP 26** | | | | | | |  |
| First amplification  Amplicon size: 864 bp | F: (5’- GGTCTAGGGCTCAACCTTACTGCT-3’)  R: (5’- CTCCAACAGCGTGTGTGTCTGTAG-3’) | | 95°C, 3 min | | | | Ankarklev et al., 2018 |
| 95°C, 20 sec | |  | |
| 55°C, 30 sec | | 35x | |  |
| 72 °C, 45 sec | |  | |  |
| 72 °C, 7 min | | | |
| Second amplification  Amplicon size: 557 bp | F: (5’-GACAACGCCTCCGTCACTTC-3’)  R: (5’-GACTCCTTGATGGCATACAACG-3’) | | 95°C, 3 min | | | | Ankarklev et al., 2018 |
| 95°C, 20 sec |  | | |
| 55°C, 30 sec | 35x | | |
| 72 °C, 45 sec |  | | |
| 72 °C, 7 min | | | |
| ***Giardia*** High cysteine protein HCMP6372 | | | | | | |  |
| First amplification  Amplicon size: 828 bp | F: (5’-GAACTTGTGTCACGCTGAATTAATACAG-3’)  R: (5’-TCGGGAACACAGACGACACCT-3’) | | 95°C, 3 min | | | | Ankarklev et al., 2018 |
| 95°C, 20 sec | |  | |
| 55°C, 30 sec | | 35x | |  |
| 72 °C, 45 sec | |  | |  |
| 72 °C, 7 min | | | |
| Second amplification  Amplicon size: 640 bp | F: (5’-GGCGGTGAGTGTGTGGAGAC-3’)  R: (5’-CTTCAGAGATGCAAGTACCATTGTTC-3’) | | 95°C, 3 min | | | | Ankarklev et al., 2018 |
| 95°C, 20 sec |  | | |
| 55°C, 30 sec | 35x | | |
| 72 °C, 45 sec |  | | |
| 72 °C, 7 min | | | |
| ***Giardia:* 6-phos-phogluconate dehydrogenase (pgd)** | | | | | | | Seabolt et al., 2021  Seabolt et al., 2021 |
| First amplification  Amplicon size: 1028 bp | F: (5’- GGR ATT RTT GCG CAR TCR CTT CC -3’)  R: (5’- CAG AGA TGT TCG YYT ACG AAA C-3’) | | 95°C, 3 min | | | |
| 94°C, 45 sec | | |  |
| 58°C, 45 sec | | | 35x |
| 72 °C, 90 sec | | |  |
| 72 °C, 7 min | | | |
| Second amplification | F: (5’- GAC TAT AGY TCR CCA ATA GGC -3’)  R: (5’- TTR TAT CTT GCA GKC AGC TGR CA-3’) | | 95°C, 3 min | | | |
| 94°C, 45 sec | | |  |
| 58°C, 45 sec | | | 35x |
| 72 °C, 90 sec | | |  |
| 72 °C, 7 min | | | |
| *Giardia***: Hypothetical protein** | | | | | | | Seabolt et al., 2021 |
| First amplification  Amplicon size: 1356 bp | F: (5’- GGT TAC YTT TCT AGG TGA YAT ATA -3’)  R: (5’- CTR CAR AAC GGW AGR CTC ARG TC-3’) | | 95°C, 3 min | | | |
| 94°C, 45 sec | |  | |
| 58°C, 45 sec | | 35x | |
| 72 °C, 90 sec | |  | |  |
| 72 °C, 7 min | | | |
| Second amplification, | F: (5’- CAG RGT GCC AAA TCT TTA CRC -3’)  R: (5’- CCC GTG AAT ACR CAY AAG CTA T-3’) | | 95°C, 3 min | | | | Seabolt et al., 2021 |
| 94°C, 45 sec |  | | |
| 58°C, 45 sec | 35x | | |
| 72 °C, 90 sec |  | | |
| 72 °C, 7 min | | | |
| *Giardia***: Phosphorylase B gamma catalytic chain kinase (phkg2)** | | | | | | | Seabolt et al., 2021 |
| First amplification  Amplicon size: 1017 bp | F: (5’- CTT GAC CTY AAT GCM TTY CTY ATG A -3’)  R: (5’- GCT YTT GTT CTG YCC AAG GCT-3’) | | 95°C, 3 min | | | |
| 94°C, 45 sec | |  | |
| 58°C, 45 sec | | 35x | |  |
| 72 °C, 90 sec | |  | |  |
| 72 °C, 7 min | | | |
| Second amplification | F: (5’- AAT CTG TCC YCT YGA GAT TGC T -3’)  R: (5’- TGA AGA GCC TCC GAG AAR TC-3’) | | 95°C, 3 min | | | | Seabolt et al., 2021 |
| 94°C, 45 sec |  | | |
| 58°C, 45 sec | 35x | | |
| 72 °C, 90 sec |  | | |
| 72 °C, 7 min | | | |
| We used the following PCR mixture for all genes: reactions were carried out in a total volume of 25 µL that included 2 µL of template DNA, 1 µL forward and 1 µL reverse primer (0.4µM concentration) and 12.5 µL of DreamTaq PCR Master Mix (2X) (Thermo Fisher Scientific) and 8.5 µL nuclease-free water. 0.2 µL Bovine serum albumin (20mg/ml) was used in the reaction for SSU, BG, TPI, and GDH gene. BSA was not included for *Cryptosporidium* and *Giardia* assemblage A and B subtyping. | | | | | | | |

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