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T_foetus_AF339736      CGGTAGGTGAACCTGCCGTTGGATCAGTTTCGTTAATAATTACAAAACA--
S_sp._KY410341        --GTAGGTGAACCTGCCGTTGGATCAGTTTCG-TACTAACTACAAAACA--
S_moskowitzi_GQ254636 --GTAGGTGAACCTGCCGTTGGATCAGTTTCG-TACTAACTACAAAACA--
T_gallinarum_JN007006 --GTAGGTGAACCTGCCGTTGGATCAGTTCTAGTTTAAATAACTAACACC
P_hominis_JN007007    --GTAGGTGAACCTGCCGTTGGATCAGTTCTAGT-TTAA---C---ACC
T_vaginalis_JN007004  --GTAGGTGAACCTGCCGTTGGATCAGTTCTAGTTTAAATAACTAACACC
                        *****
                        *   **   *   *

T_foetus_AF339736      ---TATTTTT--TTAATGTC-TATAACTATTT-ATACAAAAT-TAAACAC
S_sp._KY410341        ---TTGTTTT--TTAA-GTT-CATAAC---CT-ATACAAAACA-TAA---C
S_moskowitzi_GQ254636 ---TTGTTTT--TTAA-GTT-CATAAC---TT-ATACAAAACA-TAA---C
T_gallinarum_JN007006 AACTTCTTTTAAATTAACTTTTTTAAAC---CACATACAACAACAAA-AA
P_hominis_JN007007    AAATTCCTTTT--TTGA-----TAAAC---CA-ATACAA---TATAA-AC
T_vaginalis_JN007004  AACTTCTTTTAA-TTAA-A-C-AAAAAC---CA-ATACAAAAT-TAAA-AA
                        *   ***   ** *   *   **   *   **   *   *

T_foetus_AF339736      ATAATCT-A--AAAAATTTAGACCTTAGGCAATG----GATGTCTTGGCT
S_sp._KY410341        CGAG-CT-A--A-AAATCAAGACCTTAGGCAATGGATGGATGTCTTGGCT
S_moskowitzi_GQ254636 CGAG-CT-A--A-AAAAC-AGACCTTAGGCAATG----GATGTCTTGGCT
T_gallinarum_JN007006 ATAATAA-A--AAAAGTTAGACTCTAATCAACG----GATGTCTTGGCT
P_hominis_JN007007    CTAACCTT-A--A--TGTAAGGTCTCTAAACAATG----GATGTCTTGGCT
T_vaginalis_JN007004  CTAACCTTCAACAAAACCAAGTCTCTAAGCAATG----GATGTCTTGGCT
                        *   *   *   *   *   *   *   *   *   *   *
Forward primer GAACGTTGCATAATGCGATAAGC
T_foetus_AF339736      TCTTACACGATGAAGAACGTTGCATAATGCGATAAGCGGCTGGATTAGCT
S_sp._KY410341        CCTTACACGATGAAGAACGTTGCATAATGCGATAAGCGGCTGGAGTAGCT
S_moskowitzi_GQ254636 CCTTACACGATGAAGAACGTTGCATAATGCGATAAGCGGCTGGAGTAGCT
T_gallinarum_JN007006 CCTTACACGATGAAGAACGTTGCATAATGTGATAAGTAACCTGGAGTTGCG
P_hominis_JN007007    CCTCACACGATGAAGAACGTTGCATAATGTGATAAGTAACCTGGAGTTGCG
T_vaginalis_JN007004  CCTCACACGATGAAGAACGTTGCATAATGTGTTAAGTAACCGGAGTTGCA
                        ** ***** *   **   *   **   *   **
Probe ATCTTTGAATGCACATTGCGCGCC
T_foetus_AF339736      TTCTTTGCGACAAGTTCGATCTTTGAATGCACATTGCGCGCCGTTTT
S_sp._KY410341        AACTTTGCGACAAGTTCGATCTTTGAATGCACATTGCTCACGA-GGA
S_moskowitzi_GQ254636 AACAAATGCGACAAGTTCGATCTTTGAATGCACATTGCGCAATACGTA
T_gallinarum_JN007006 AACATTGTGACAAGTT-AATCTTTGAATGCAAATGCGCGTACCTTT
P_hominis_JN007007    AACATTGTGACAAGTT-AATCTTTGAATGCAAATGCGCATACC-TG
T_vaginalis_JN007004  AACATCATGACAGGTT-AATCTTTGAATGCAAATGCGCTAAACTCG
                        *   ****   ** ***** *   **   *
Reverse primer AGCTT GCTAGAACACGCATATATGTT
T_foetus_AF339736      AGCTT--GCTAGAACACGCATATATGTTACAGTAACCCATATTAATTTAATAC
S_sp._KY410341        GGCTT--GAAACGGAGTGAATGTATGAAGTAGTAACC-A-TTTG-TATTAT--
S_moskowitzi_GQ254636 TACTT--GTATACGTATGCATATATGAAGTAGTAACC-A-ATTA-CATTCT--
T_gallinarum_JN007006 ACCTTCGGGTATGTTGTCATGTATGCCACAGTACTGCATAAAA-TAATAT--
P_hominis_JN007007    AATTT--A-TTC-AGATGCATATATATTCTAGTACTACATCTTT-TAAT---
T_vaginalis_JN007004  ATCTC--GGTCG-AGAAGCATGGGTGTGACAGTACTACATCTTT-TATAAT--
                        *   **   *   ***   *

T_foetus_AF339736      CAAATTCCTCTTTTTAAGCAAAGAGCGAAAAACAATATGTATTAAACAAA
S_sp._KY410341        --AATTCGCTTTTCTA-TCAAA-GCGTGAAAAACT-CTAATT---ACAAAA
S_moskowitzi_GQ254636 --AATTCGCTTTTCTA-TCAAA-GCGGAAAAAACC-ATAATC---AAGAAT
T_gallinarum_JN007006 -ATATTCCTCTTTTC-GCAAAGAAGCAAATATTTAAGCA-AAAAGAT
P_hominis_JN007007    -A-ATTCTTCTTATA---AAAGAAGCAAATAAC-TAAAAGCA---AA--C
T_vaginalis_JN007004  -A-ATTCTTATTCTAAGCGAATAAGTAAATAATATATAAGAC-AAACAAC
                        ****   ** *   ** *   ** *   *

T_foetus_AF339736      AG-GGTTCTGTCTCATATAGGAAGACCCGCTGAACTGAAGCA
S_sp._KY410341        TT-GGTTCTGCGACATGCAGGAAGACCCGCTGAACTGA---A
S_moskowitzi_GQ254636 TC-GGTTCTTCAACATATAGGAAGACCCGCTGAACTGA---A
T_gallinarum_JN007006 ACGTAGTCTGCGACATACAGGAAGACCCGCTGAACTGA---A
P_hominis_JN007007    ACGTAGTCTGGGATATGTAGGAAGACCCGCTGAACTGA---A
T_vaginalis_JN007004  ACGTAGTCTGCCATACGCAGGAAGACCCGCTGAACTGA---A
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**Supplementary Figure 1.** Alignment of *Tritrichomonas foetus* assay primers and probe sequences with *T. foetus*, order Tritrichomonadida, and order Trichomonadida sequences. Multiple sequence alignment was performed by Clustal W (<https://goo.gl/XNwwhC>). GenBank accessions are provided after organism's name; "S\_sp." denotes *Simplicimonas* sp. Asterisks denote identical nucleotides among the aligned sequences. Primers and probe sequences are provided above the *T. foetus* sequence.