

Conventional sequences

Leishmania colombiensis AGGCTACCGTTTCGGCTTTTGTTGGTTTTAAAGGTTATTGGAGATTATGGAGCTGTGCGACAAGCGTTTCCCATCGCAACTTCGGTTCCGGTGTGTG
 Leishmania naiffi AGGCTACCGTTTCGGCTTTTGTTGGTTTTAAAGGTTATTGGAGATTATGGAGCTGTGCGACAAGCGTTTCCCATCGCAACTTCGGTTCCGGTGTGTG
 Leishmania panamensis AGGCTACCGTTTCGGCTTTTGTTGGTTTTAAAGGTTATTGGAGATTATGGAGCTGTGCGACAAGCGTTTCCCATCGCAACTTCGGTTCCGGTGTGTG
 Leishmania guyanensis AGGCTACCGTTTCGGCTTTTGTTGGTTTTAAAGGTTATTGGAGATTATGGAGCTGTGCGACAAGCGTTTCCCATCGCAACTTCGGTTCCGGTGTGTG
 Leishmania braziliensis AGGCTACCGTTTCGGCTTTTGTTGGTTTTAAAGGTTATTGGAGATTATGGAGCTGTGCGACAAGCGTTTCCCATCGCAACTTCGGTTCCGGTGTGTG
 Leishmania lainsoni AGGCTACCGTTTCGGCTTTTGTTGGTTTTAAAGGTTATTGGAGATTATGGAGCTGTGCGACAAGCGTTTCCCATCGCAACTTCGGTTCCGGTGTGTG
 Leishmania shawi AGGCTACCGTTTCGGCTTTTGTTGGTTTTAAAGGTTATTGGAGATTATGGAGCTGTGCGACAAGCGTTTCCCATCGCAACTTCGGTTCCGGTGTGTG
 Leishmania amazonensis AGGCTACCGTTTCGGCTTTTGTTGGTTTTAAAGGTTATTGGAGATTATGGAGCTGTGCGACAAGCGTTTCCCATCGCAACTTCGGTTCCGGTGTGTG
 Leishmania mexicana AGGCTACCGTTTCGGCTTTTGTTGGTTTTAAAGGTTATTGGAGATTATGGAGCTGTGCGACAAGCGTTTCCCATCGCAACTTCGGTTCCGGTGTGTG
 Leishmania aethiopica AGGCTACCGTTTCGGCTTTTGTTGGTTTTAAAGGTTATTGGAGATTATGGAGCTGTGCGACAAGCGTTTCCCATCGCAACTTCGGTTCCGGTGTGTG
 Leishmania donovani AGGCTACCGTTTCGGCTTTTGTTGGTTTTAAAGGTTATTGGAGATTATGGAGCTGTGCGACAAGCGTTTCCCATCGCAACTTCGGTTCCGGTGTGTG
 Leishmania infantum AGGCTACCGTTTCGGCTTTTGTTGGTTTTAAAGGTTATTGGAGATTATGGAGCTGTGCGACAAGCGTTTCCCATCGCAACTTCGGTTCCGGTGTGTG
 Leishmania major AGGCTACCGTTTCGGCTTTTGTTGGTTTTAAAGGTTATTGGAGATTATGGAGCTGTGCGACAAGCGTTTCCCATCGCAACTTCGGTTCCGGTGTGTG
 Leishmania tropica AGGCTACCGTTTCGGCTTTTGTTGGTTTTAAAGGTTATTGGAGATTATGGAGCTGTGCGACAAGCGTTTCCCATCGCAACTTCGGTTCCGGTGTGTG

Bisulphite converted sequences

Leishmania colombiensis AGGCTATTGTTTCGGCTTTTGTTGGTTTTAAAGGTTATTGGAGATTATGGAGCTGTGCGACAAGCGTTTCCCATCGCAACTTCGGTTCCGGTGTGTG
 Leishmania naiffi AGGCTATTGTTTCGGCTTTTGTTGGTTTTAAAGGTTATTGGAGATTATGGAGCTGTGCGACAAGCGTTTCCCATCGCAACTTCGGTTCCGGTGTGTG
 Leishmania panamensis AGGCTATTGTTTCGGCTTTTGTTGGTTTTAAAGGTTATTGGAGATTATGGAGCTGTGCGACAAGCGTTTCCCATCGCAACTTCGGTTCCGGTGTGTG
 Leishmania guyanensis AGGCTATTGTTTCGGCTTTTGTTGGTTTTAAAGGTTATTGGAGATTATGGAGCTGTGCGACAAGCGTTTCCCATCGCAACTTCGGTTCCGGTGTGTG
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 Leishmania lainsoni AGGCTATTGTTTCGGCTTTTGTTGGTTTTAAAGGTTATTGGAGATTATGGAGCTGTGCGACAAGCGTTTCCCATCGCAACTTCGGTTCCGGTGTGTG
 Leishmania shawi AGGCTATTGTTTCGGCTTTTGTTGGTTTTAAAGGTTATTGGAGATTATGGAGCTGTGCGACAAGCGTTTCCCATCGCAACTTCGGTTCCGGTGTGTG
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 Leishmania mexicana AGGCTATTGTTTCGGCTTTTGTTGGTTTTAAAGGTTATTGGAGATTATGGAGCTGTGCGACAAGCGTTTCCCATCGCAACTTCGGTTCCGGTGTGTG
 Leishmania aethiopica AGGCTATTGTTTCGGCTTTTGTTGGTTTTAAAGGTTATTGGAGATTATGGAGCTGTGCGACAAGCGTTTCCCATCGCAACTTCGGTTCCGGTGTGTG
 Leishmania donovani AGGCTATTGTTTCGGCTTTTGTTGGTTTTAAAGGTTATTGGAGATTATGGAGCTGTGCGACAAGCGTTTCCCATCGCAACTTCGGTTCCGGTGTGTG
 Leishmania infantum AGGCTATTGTTTCGGCTTTTGTTGGTTTTAAAGGTTATTGGAGATTATGGAGCTGTGCGACAAGCGTTTCCCATCGCAACTTCGGTTCCGGTGTGTG
 Leishmania major AGGCTATTGTTTCGGCTTTTGTTGGTTTTAAAGGTTATTGGAGATTATGGAGCTGTGCGACAAGCGTTTCCCATCGCAACTTCGGTTCCGGTGTGTG
 Leishmania tropica AGGCTATTGTTTCGGCTTTTGTTGGTTTTAAAGGTTATTGGAGATTATGGAGCTGTGCGACAAGCGTTTCCCATCGCAACTTCGGTTCCGGTGTGTG

Figure S1. Conventional and bisulphite converted alignments for the 18S rDNA gene.

Table 1. Conventional and bisulphite converted primer and probe designs for the novel assay.

	Conventional Sequence	Tm	Bisulphite Converted Sequence	Tm
Forward Primer	CTACCGTTTCGGCTTTTG	60.9	TTATTGTTTTGGTTTTTG	50.8
Reverse Primer	TCCCATCGCAACCTCGGTTC	71.6	TTTTATTGTAATTTTGGTTT	50.8
Probe	GGAGATTATGGAGCTGTGCGACA	69.3	GGAGATTATGGAGTTGTGTGATA	59.4

>gi|261499643|gb|GQ920678.1| Leishmania aethiopica isolate 1214 18S ribosomal RNA gene, nete sequence

>gi|1302626|gb|U21687.1|LAU21687 Leishmania amazonensis 18S ribosomal RNA, intergenic region, promoter region, and external transcribed spacer, partial sequence

>gi|577861717|emb|HG512966.1| *Leishmania braziliensis* genomic DNA containing 18S rRNA gene, ITS1 and 5.8S rRNA gene, strain MHOM/PE/03/LH2511
>gi|656462896|gb|KF302750.1| *Leishmania colombiensis* isolate IGOM/PA/1985/E582.34 18S ribosomal RNA gene, partial sequence
>gi|79677117|emb|AJ634376.1| *Leishmania donovani* 18S rRNA gene (partial), 5.8S rRNA gene, 28S rRNA gene (partial), ITS1 and ITS2, isolate MHOM/IN/00/DEVI
>gi|254847849|gb|GQ332358.1| *Leishmania guyanensis* 18S ribosomal RNA gene, complete sequence; and internal transcribed spacer 1, partial sequence
>gi|891862543|ref|XR_001203206.1| *Leishmania infantum* JPCM5 18S ribosomal RNA (SSU) RNA rRNA
>gi|575527028|gb|KF041805.1| *Leishmania lainsoni* isolate IOCL 1023 18S ribosomal RNA gene, partial sequence
>gi|577861714|emb|HG512963.1| *Leishmania major* genomic DNA containing 18S rRNA gene, ITS1 and 5.8S rRNA gene, strain MHOM/BF/2004/REN04-8 (LEM4886)
>gi|577861716|emb|HG512965.1| *Leishmania mexicana* genomic DNA containing 18S rRNA gene, ITS1 and 5.8S rRNA gene, strain MHOM/PE/02/LH2312
>gi|575527030|gb|KF041807.1| *Leishmania naiffi* isolate IOCL 1365 18S ribosomal RNA gene, partial sequence
>gi|254847853|gb|GQ332362.1| *Leishmania panamensis* 18S ribosomal RNA gene, complete sequence; and internal transcribed spacer 1, partial sequence
>gi|575527031|gb|KF041808.1| *Leishmania shawi* isolate IOCL 1545 18S ribosomal RNA gene, partial sequence
>gi|577861678|emb|HG512927.1| *Leishmania tropica* genomic DNA containing 18S rRNA gene, ITS1 and 5.8S rRNA gene, strain MHOM/EG/90/LPN65 (LEM2001)