

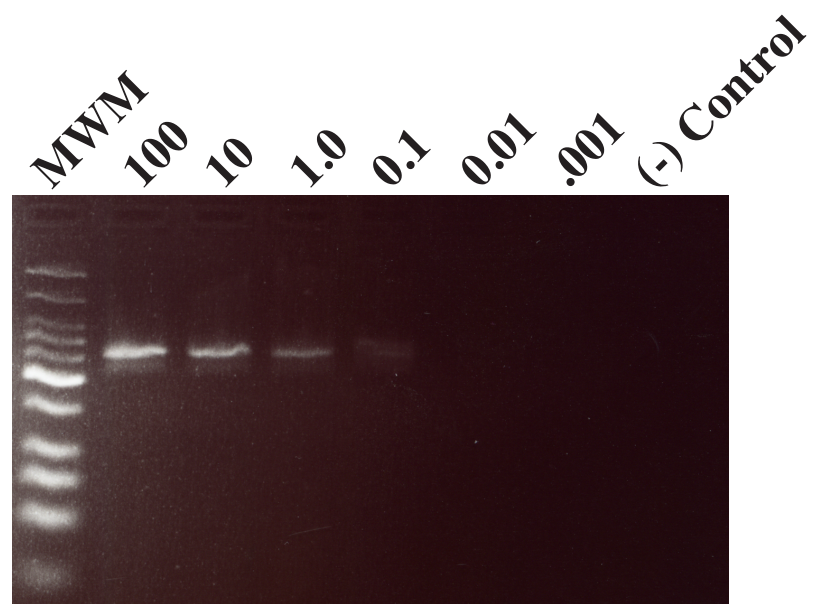
In Silico Identification of Novel Biomarkers and Development of New Rapid Diagnostic Tests for the Filarial Parasites *Mansonella perstans* and *Mansonella ozzardi*

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Supplementary Fig. 1



Supplementary Fig. 2B

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midge_29AA -----AAGGATCATTAAACGAGCTTCCAAACAAATACATAATAACAAATGAAATGTTATCCATAAATTATTATTACCAATTCACCTTTTATTTAGCAACATGCATGAGTATATACATATATA 125
midge_29L -----AAGGATCATTAAACGAGCTTCCAAACAAATACATAATAACAAATGAAATGTTATCCATAAATTATTATTACTATTCACCTTTTATTTAGCAACATGCATGAGTATATACATATATA 125
DQ995498.1 GGTGAACCTGCGGAAAGGATCATTAAACGAGCTTCCAAACAAATACATAATAACAAATGAAATGTTATCCATAAATTATTATTACTATTCACCTTTTATTTAGCAACATGCATGAGTATATACATATATA 125
midge_29A -----GGAAGGATCATTAAACGAGCTTCCAAACAAATACATAATAACAAATGAAATGTTATCCATAAATTATTATTACTATTCACCTTTTATTTAGCAACATGCATGAGTATATACATATATA 125
midge_29Q -----NGGAAGGATCATTAAACGAGCTTCCAAACAAATACATAATAACAAATGAAATGTTATCCATAAATTATTATTACTATTCACCTTTTATTTAGCAACATGCATGAGTATATACATATATA 125

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midge_29AA TAGTTGCTTTGCTATTATTTAATATTAGTGAAATAGTTAAATAAATAATTGATACAACTGAATTAACGGTGATATTGTTGGTGTCTATACCTTTATCCAAATTTATCGCCTAAACCGTCGATAATGAT 250
midge_29L TAGTTGCTTTGCTATTATTTAATATTAGTGAAATAGTTAAATAAATAATTGATACAACTGAATTAACGGTGATATTGTTGGTGTCTATACCTTTATCCAAATTTATCGCCTAAACCGTCGATAATGAT 250
DQ995498.1 TAGTTGCTTTGCTATTATTTAATATTAGTGAAATAGTTAAATAAATAATTGATACAACTGAATTAACGGTGATATTGTTGGTGTCTATACCTTTATCCAAATTTATCGCCTAAACCGTCGATAATGAT 250
midge_29A TAGTTGCTTTGCTATTATTTAATATTAGTGAAATAGTTAAATAAATAATTGATACAACTGAATTAACGGTGATATTGTTGGTGTCTATACCTTTATCCAAATTTATCGCCTAAACCGTCGATAATGAT 250
midge_29Q TAGTTGCTTTGCTATTATTTAATATTAGTGAAATAGTTAAATAAATAATTGATACAACTGAATTAACGGTGATATTGTTGGTGTCTATACCTTTATCCAAATTTATCGCCTAAACCGTCGATAATGAT 250

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midge_29AA GAAGATAAAGCGGATAGCTTAAATTAATTTTTTATGAAAAATTAATTAAGTAGACTTAATAAGCATTATGCTAAATATGCTACCAACAAATAAATACACACATACATATACAAATTTGAATTTATGA 375
midge_29L GAAAAATAAAGCGGATAGCTTAAATTAATTTTTTATGAAAAATTAATTAAGTAGACTTAATAAGCATTATGCTAAATATGCTACCAACAAATAAATACACACATACATATACAAATTTGAATTTATGA 375
DQ995498.1 GAAGATAAAGCGGATAGCTTAAATTAATTTTTTATGAAAAATTAATTAAGTAGACTTAATAAGCATTATGCTAAATATGCTACCAACAAATAAATACACACATACATATACAAATTTGAATTTATGA 375
midge_29A GAAGATAAAGCGGATAGCTTAAATTAATTTTTTATGAAAAATTAATTAAGTAGACTTAATAAGCATTATGCTAAATATGCTACCAACAAATAAATACACACATACATATACAAATTTGAATTTATGA 375
midge_29Q GAAGATAAAGCGGATAGCTTAAATTAATTTTTTATGAAAAATTAATTAAGTAGACTTAATAAGCATTATGCTAAATATGCTACCAACAAATAAATACACACATACATATACAAATTTGAATTTATGA 375

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midge_29AA AAAAAATATTAAAGAAATTTTTTAACTCTTAGCGGTGGATCACTTGGCTCATGGATCGATGAAGAACGCGACTAGCTGCGATAAAATAGTGGCAATTG----- 484
midge_29L AAAAAATATTAAAGAAATTTTTTAACTCTTAGCGGTGGATCACTTGGCTCATGGATCGATGAAGAACGCGACTAGCTGCGATAAAATAGTGGCAATTG----- 484
DQ995498.1 AAAAAATATTAAAGAAATTTTTTAACTCTTAGCGGTGGATCACTTGGCTCATGGATCGATGAAGAACGCGACTAGCTGCGATAAAATAGTGGCAATTGCGACGCATTGAG 484
midge_29A AAAAAATATTAAAGAAATTTTTTAACTCTTAGCGGTGGATCACTTGGCTCATGGATCGATGAAGAACGCGACTAGCTGCGATAAAATAGTGGCAATT----- 484
midge_29Q AAAAAATATTAAAGAAATTTTTTAACTCTTAGCGGTGGATCACTTGGCTCATGGATCGATGAAGAACGCGACTAGCTGCGATAAAATAGTGGCAATT----- 484

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Supplementary Fig. 3

(A)

		Microscopy results = Imperfect gold standard (S)			
		S+	S-		
LAMP assay = New test (N)	N+	9	1	10	→
	N-	0	10	10	
		9	11	20	

		ITS1 nested-PCR results = Imperfect Resolver (R)			
		S+	S-,R+	S-,R-	
LAMP assay = New test (N)	N+	9+1 = 10	1-1 = 0	10	→
	N-	0+0 = 0	10-0 = 10	10	
		10	10	20	

(B)

a = 9
b = 1
c = 0
d = 10



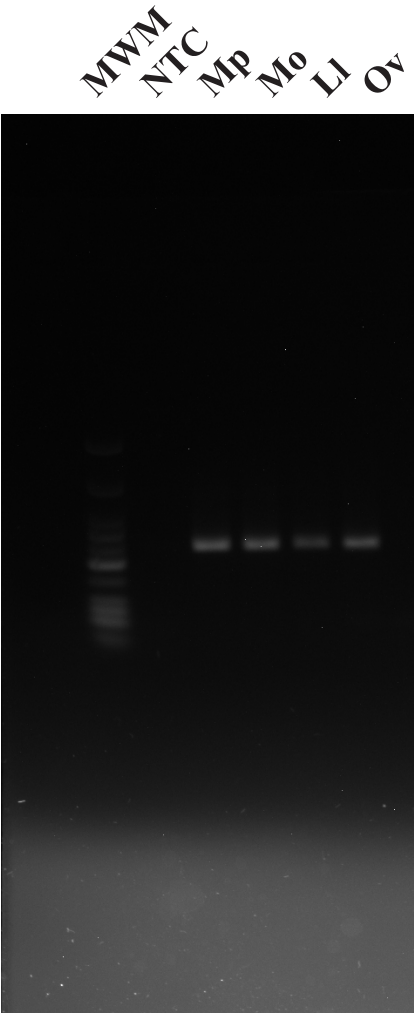
$a' = 1$
 $\Rightarrow a + a' = 9 + 1 = 10$
 $\Rightarrow b - a' = 1 - 1 = 0$
 $c' = 0$
 $\Rightarrow c + c' = 0 + 0 = 0$
 $\Rightarrow d - c' = 10 - 0 = 10$

Sensitivity = $(a + a') / ((a+c) + (a' + c'))$
 $= (9 + 1) / ((9+0) + (1 + 0)) = 10/10 = 100\%$

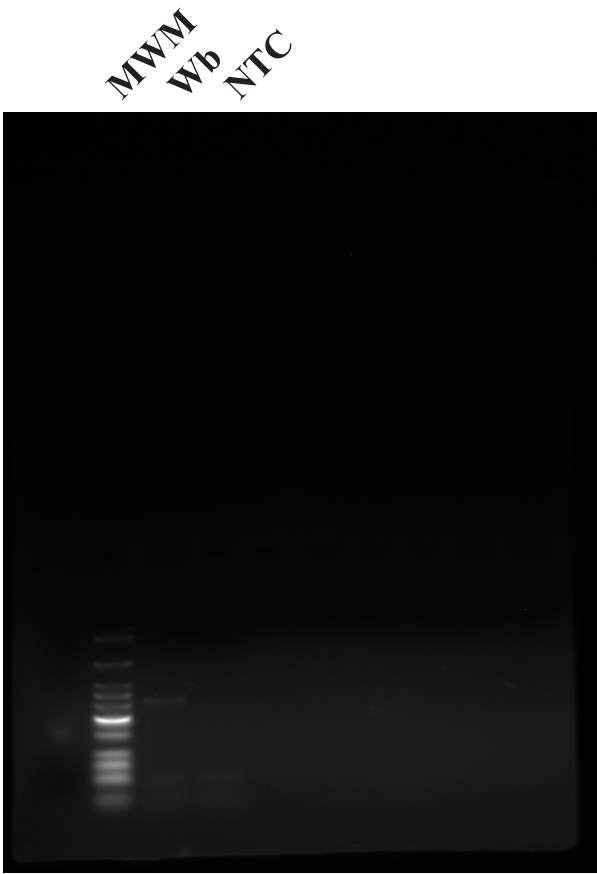
Specificity = $(d - c') / ((b + d) - (a' + c'))$
 $= (10 - 0) / ((1 + 10) - (1 + 0)) = 10/10 = 100\%$

Supplementary Fig. 4

A.



B.



Supplementary Fig. 1. Sensitivity of *Mansonella* nested-PCR assay. *M. perstans* DNA ranging from 100-0.001 pg per reaction, was amplified by ITS1 nested PCR³¹. A negative (-) control containing 1 ng of HeLa DNA (New England Biolabs) was included. The Low Molecular Weight DNA ladder (New England Biolabs) was used as the MWM.

Supplementary Fig. 2. Sequencing is required to distinguish ITS1 amplicons of *M. perstans* from *M. ozzardi*. (A) Alignment of *M. perstans* (GenBank ID: DQ995498.1) and *M. ozzardi* (GenBank ID: AF228559.1) sequences shows that the published³¹ primer sequences ITS1-F and ITS1-R (blue arrows), and MpF1 and MpR1 (green arrows) can amplify the ITS1 region of both species necessitating sequencing to correctly identify the species. (B) Multiple sequence alignment showing > 99.5 % identity between the reference ITS1 sequence from *M. perstans* (GenBank ID DQ995498.1) and the ITS1 region sequenced from the 4 midge samples that tested positive by ITS1 nested-PCR but negative by Mp419 colorimetric LAMP.

Supplementary Fig. 3. The two-stages of the composite reference standard (CRS) test for evaluating the performance of Mp419 LAMP assay on human samples. (A) In this CRS test, the LAMP assay is the new assay (N), microscopy is designated as the “imperfect standard” (S), and ITS1 nested-PCR is designated as the “imperfect resolver” (R). (B) Calculations of sensitivity and specificity. The changes introduced by R are a’ and c’. For more details on methodology and terminology, see Hess *et al.*⁷²

Supplementary Fig. 4. The original uncropped gels from which Fig. 4C was derived. The actin amplicons for *M. perstans* (Mp), *M. ozzardi* (Mo), *L. loa* (Ll), *O. volvulus* (Ov) and *W. bancrofti* (Wb) are shown in parts A and B respectively. The low molecular weight DNA ladder (New

England Biolabs) was used as the molecular weight marker (MWM). Water was substituted for DNA in the non-template controls (NTC).