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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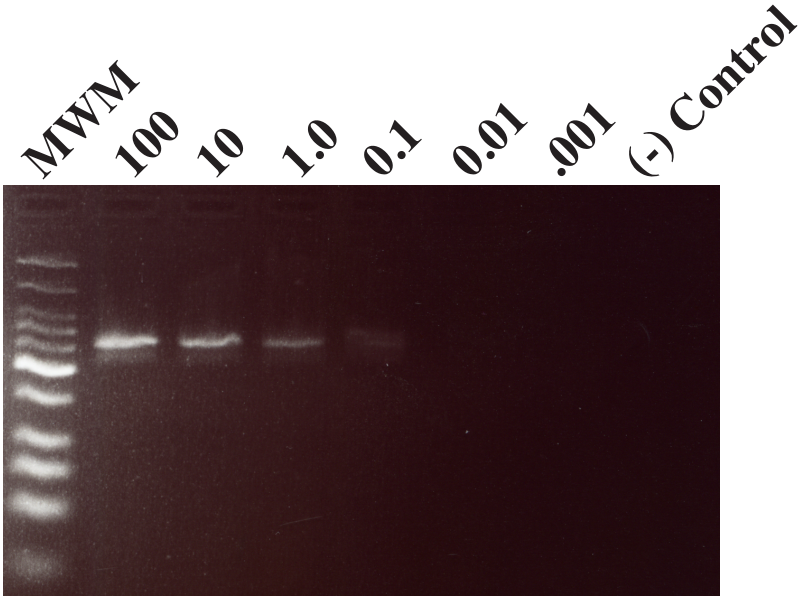
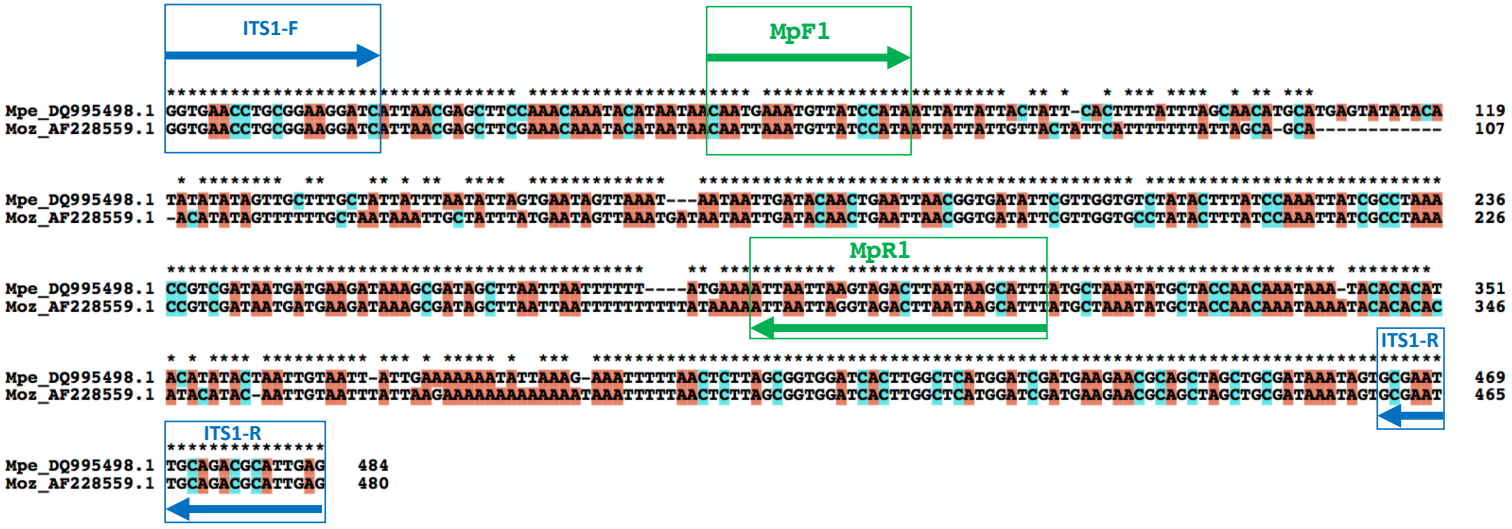
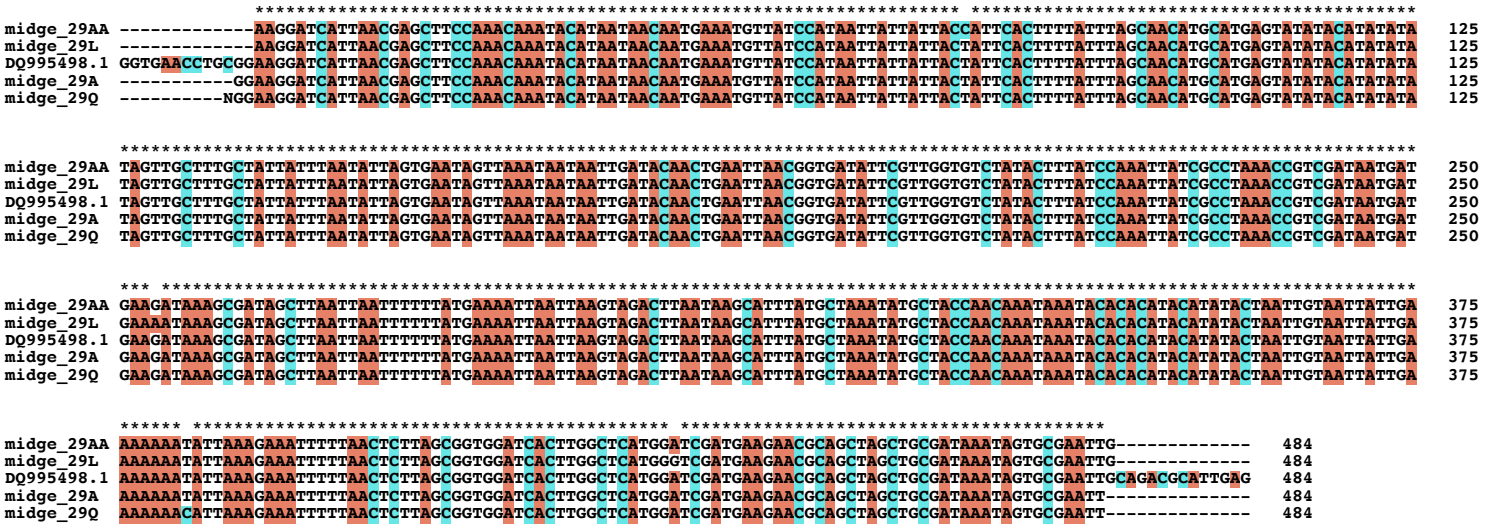
Supplementary Fig. 1



Supplementary Fig. 2A



Supplementary Fig. 2B



Supplementary Fig. 3

(A)

(Stage 1)

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

(Stage 2)

		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
=> a + a’ = 9 +1 = 10
=> b – a’ = 1 - 1 = 0

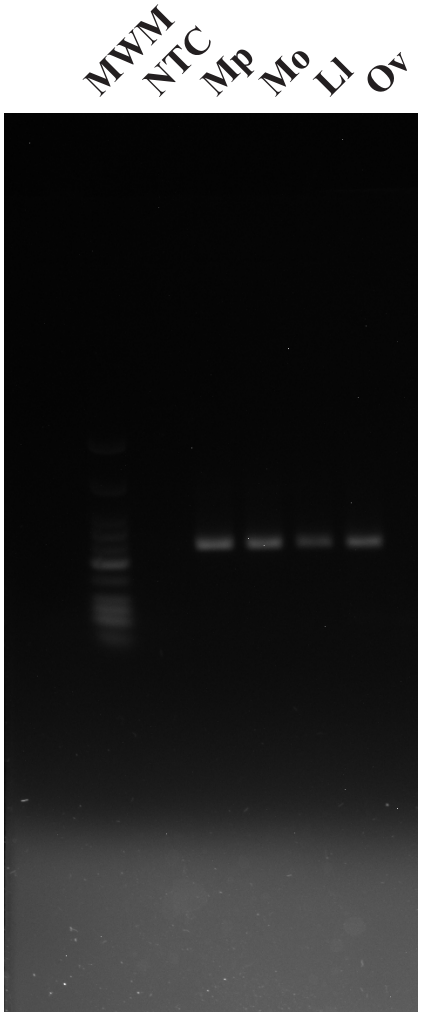
c’ = 0
=> c + c’ = 0 + 0 = 0
=> d – c’ = 10 - 0 = 10

Sensitivity = (a + a’) / ((a+c) + (a’ + c’))
= (9 + 1) / ((9+0) + (1 + 0)) = 10/10 = 100%

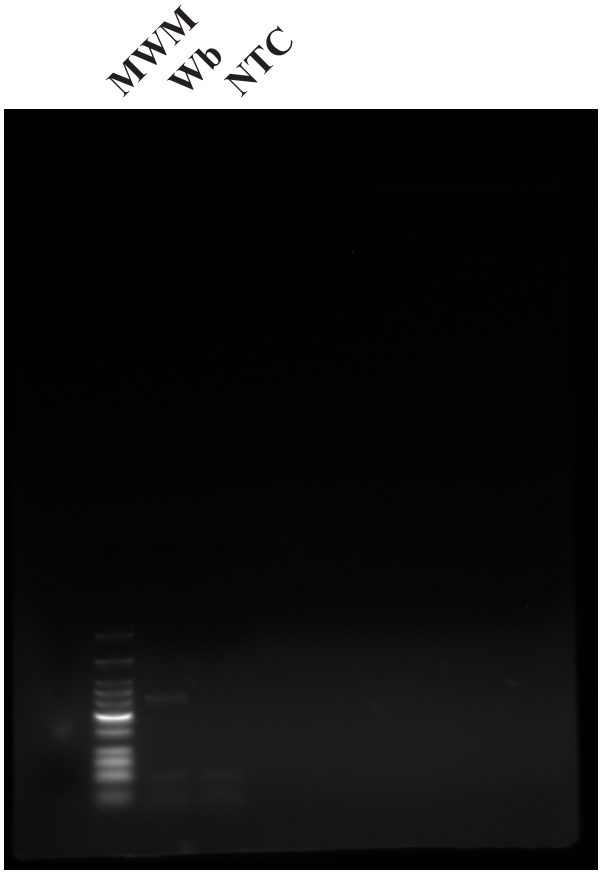
Specificity = (d – c’) / ((b + d) – (a’ + c’))
= (10 – 0) / ((1 + 10) – (1 + 0)) = 10/10 = 100%

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 PCR[31]. 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 published[31] 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).