Genome filtering identifies species-specific DNA biomarkers for *Mansonella perstans* and *Mansonella ozzardi* which enable differentiation of these closely related species and other co-endemic filarial parasites

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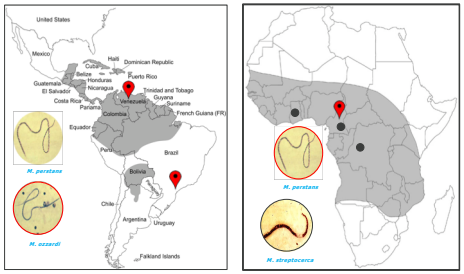
Introduction to Mansonelliasis

- Caused by 3 parasites: *Mansonella perstans*, *M. ozzardi* & *M. streptocerca*.
- Primary Insect Vector: *Culicoides* (biting midges).
  - *M. ozzardi* also spread by *Simulium* (black flies).
- No distinct, specific clinical consequences for *Mansonella* infections.
  - Immunosuppression caused by parasitic infection may lead to worsening of other medical conditions.
- Anti-helminthic treatment is complicated:
  - Not all species respond to ivermectin.
  - Benzimidazoles & DEC often employed.
- *Mansonella* patients are often co-infected with multiple filarial parasites including *Onchocerca volvulus*, *Wuchereria bancrofti*, and *Loa loa*.
- Need improved diagnostic tools.

Regions where Mansonelliasis is Endemic

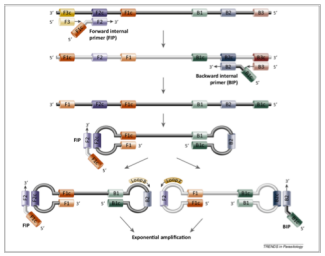


Grey shading represents regions endemic for Mansonelliasis

Origin of parasite sample

Region co-endemic for *M. perstans* & *M. streptocerca*

Loop-Mediated Isothermal Amplification (LAMP) is Ideal for Field Use



- Set of 4 primers (F3, FIP, B3 & BIP) recognize 6 distinct sequences on target DNA. Optional Loop primers speed up amplification.
- Single step isothermal reaction requiring a simple water bath.
- Highly sensitive and specific.
- Requires strand displacing polymerase (*Bst*).
- Rapid (~ 30-60 min) compared to PCR.
- Multiple direct methods for easy visualization of results.

Bioinformatic Pipeline Identifies New Diagnostic Biomarkers for *M. perstans* and *M. ozzardi*

Constructed & sequenced Illumina libraries for each species

→ Obtained ~ 227 million *M. perstans* and ~ 279 million *M. ozzardi* reads

→ Remove contaminating human reads

→ Collect reads that generate high frequency k-mers (1,000x to 100,000x)

→ Assemble reads into repeats using Trinity[1]

→ Remove sequences present in other filarial genomes:

- *Brugia malayi*
- *Onchocerca volvulus*
- *Loa loa*
- *Wuchereria bancrofti*

BLASTN search NCBI nr/nt
e-value <= 1E-10

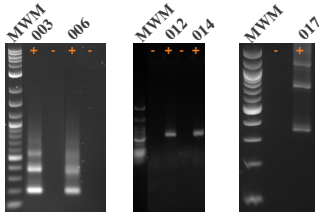
→ Identified 109 *M.perstans* & 5 *M.ozzardi* specific repeats

→ Ranked repeats:
- %GC
- length
- # of aligned reads to generate consensus (a proxy for copy #)

→ Chose 4 *M. perstans* & 1 *M. ozzardi* highly ranked repeats for LAMP assay development

[1] Grabherr *et. al.* (2011) *Nature Biotechnology* 29:644-654

Validation of Bioinformatically Identified Repeats by PCR



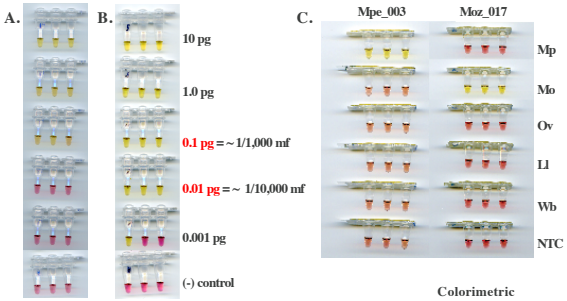
PCR of candidate biomarkers from *M. perstans* (003, 006, 012 and 014) or *M. ozzardi* (017) DNA. Ladder-like arrays (003, 006 and 017) suggest repeats are organized tandemly in the genome whereas single bands (012, 014) suggest a dispersed organization. + = DNA; - = Non-template control; MWM = molecular weight marker.

Candidate Biomarker Evaluation: Repeat Characteristics and LAMP Assay Results

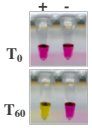
Target	[1]# Reads aligned for consensus	%GC	Consensus sequence length (bps)	Repeat type	[2]Colorimetric LAMP Optimal conditions	[2]Colorimetric LAMP Sensitivity (pg)	[2]Colorimetric LAMP Species specific
Mpe_003	80, 315	33	366	tandem	63ºC, 60 min	0.1	Yes
Mpe_006	111, 796	31	579	tandem	63ºC, 60 min	0.1	Yes
Mpe_012	21, 974	29	338	dispersed	63ºC, 60 min	1000	Yes
Mpe_014	13, 223	29	318	dispersed	61ºC, 60 min	10	Yes
Moz_017	10, 000	42	303	tandem	63ºC, 20 min	0.01	Yes

[1] # reads aligned to form the consensus sequence is used as a proxy for copy number.
[2] Multiple LAMP primer sets were designed for each target. The results generated by the best primer set are presented.

The *Mansonella* LAMP Assays are Sensitive and Specific



Colorimetric readout



The primer set targeting Mpe_003 detects as little as 0.1 pg *M. perstans* DNA (A) whereas the primer set targeting Moz_017 can detect as little as 0.01 pg of *M. ozzardi* DNA (B). The Mpe_003 and Moz_017 LAMP primer sets are specific for *M. perstans* (Mp) and *M. ozzardi* (Mo) DNA respectively. They do not cross react with DNA from the non-specific *Mansonella* species or with *O. volvulus* (Ov), *L. loa* (Ll) or *W. bancrofti* (Wb) DNA (C).

Validation on Patient and Insect Samples

Table 1. Detection of *M. perstans* in experimentally infected *C. milnei*. Comparison of the performance of ITS1 nested-PCR and colorimetric Mpe_003 LAMP.

C. milnei Infection Status	Sample Size	*Mansonella* Nested-PCR Positive	*M. perstans* LAMP Positive
FED ON VOLUNTEER: potentially infected	36	14	10
UNFED: presumed uninfected	36	1	0

Table 2. Detection of *M. perstans* in patient samples. Comparison of the performance of microscopy, ITS1 nested-PCR and colorimetric Mpe_003 LAMP.

Patient Infection Status [a]	Sample Size	*Mansonella* Nested-PCR Positive	*M. perstans* LAMP Positive
Mf POSITIVE	9	9	9
Mf NEGATIVE	1	1	1

[a] As determined by microscopic examination of patient samples.

Table 3. Detection of *M. ozzardi* in patient samples. Comparison of the performance of microscopy/ITS-2 qPCR, ITS1 nested-PCR and Moz_017 LAMP.

Patient Infection Status [b]	Sample Size	*Mansonella* Nested-PCR Positive	*M. ozzardi* LAMP Positive
Mf / qPCR POSITIVE	51	Not Evaluated	51
Mf / qPCR NEGATIVE	33	8	8

[b] As determined by microscopy and ITS-2 qPCR (Lima et. al. (2018) PLOS NTD 12:e0006327)

Summary

- Using a bioinformatic filtering approach, new diagnostic biomarkers for *M. perstans* and *M. ozzardi* were identified.
- Developed sensitive and species-specific LAMP assays targeting these new biomarkers.
- Validated these new LAMP assays on both patient and insect samples.
- These new field deployable assays will assist with the effort to better understand the global burden of Mansonelliasis.

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