GROUP 3: Identification of Leishmania by kDNA qPCR & HRM

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MeBoP 2017

qPCR/KDNA

INTRODUCTION

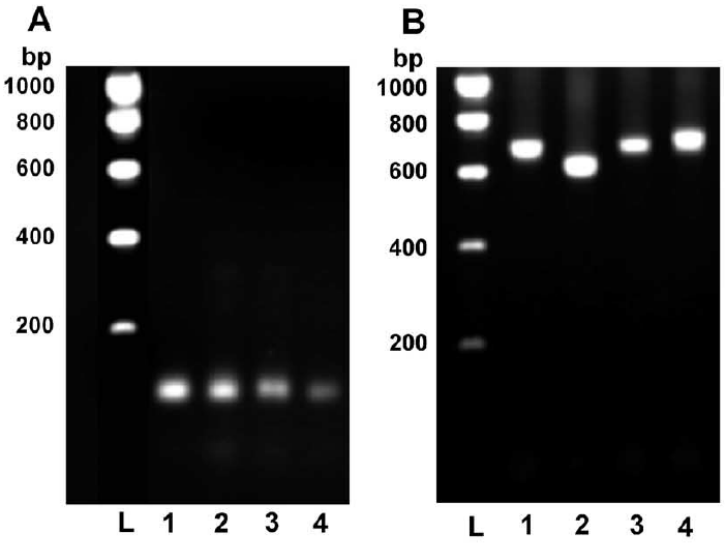
- Quantitative assessment of the Leishmania load in host tissues has been proposed to be useful in monitoring the response to antileishmanial therapy
- The traditional method of quantification of Leishmania in host tissues is the limiting dilution assay (LDA)

  arduous and time-consuming;

  it depends on sterile conditions

  highly trained personnel and can be applied only with fresh samples with relatively high parasite loads because of its low sensitivity
- Formal demonstration requires highly sensitive detection and accurate quantification of Leishmania in human blood, lesional tissue or vector
- The study used real-time PCR (qPCR) assay to target Leishmania minicircle kinetoplast DNA (kDNA).
- Melting curves analysis offers a rapid alternative for identification of species in diagnostic or epidemiological studies of leishmaniasis or asymptomatic parasitism

HRM Mechanism



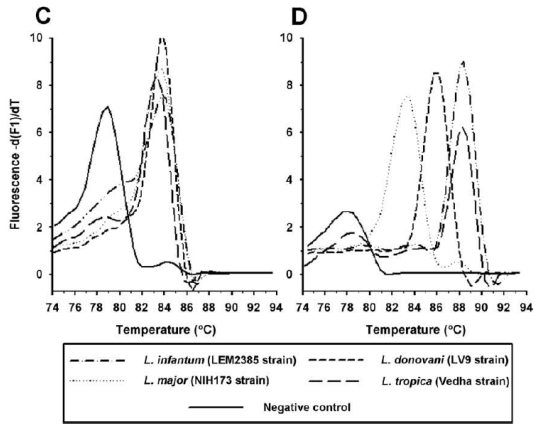
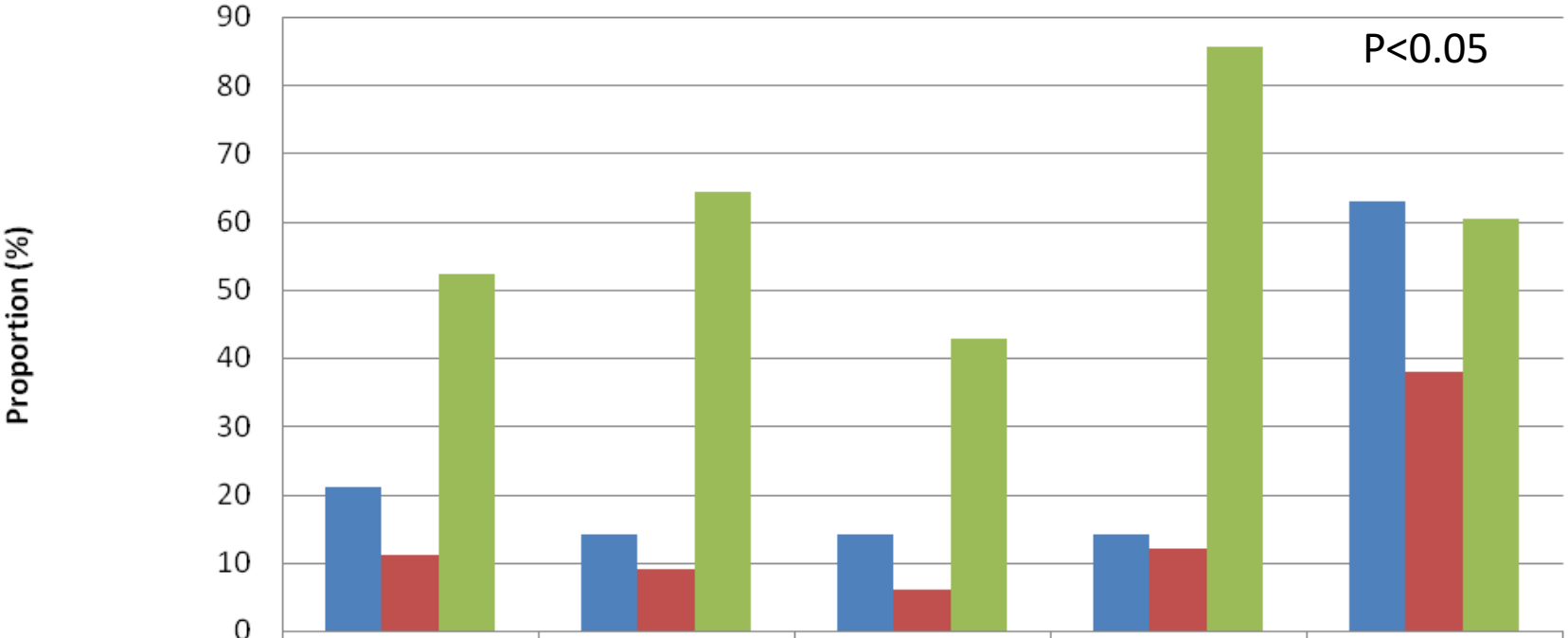


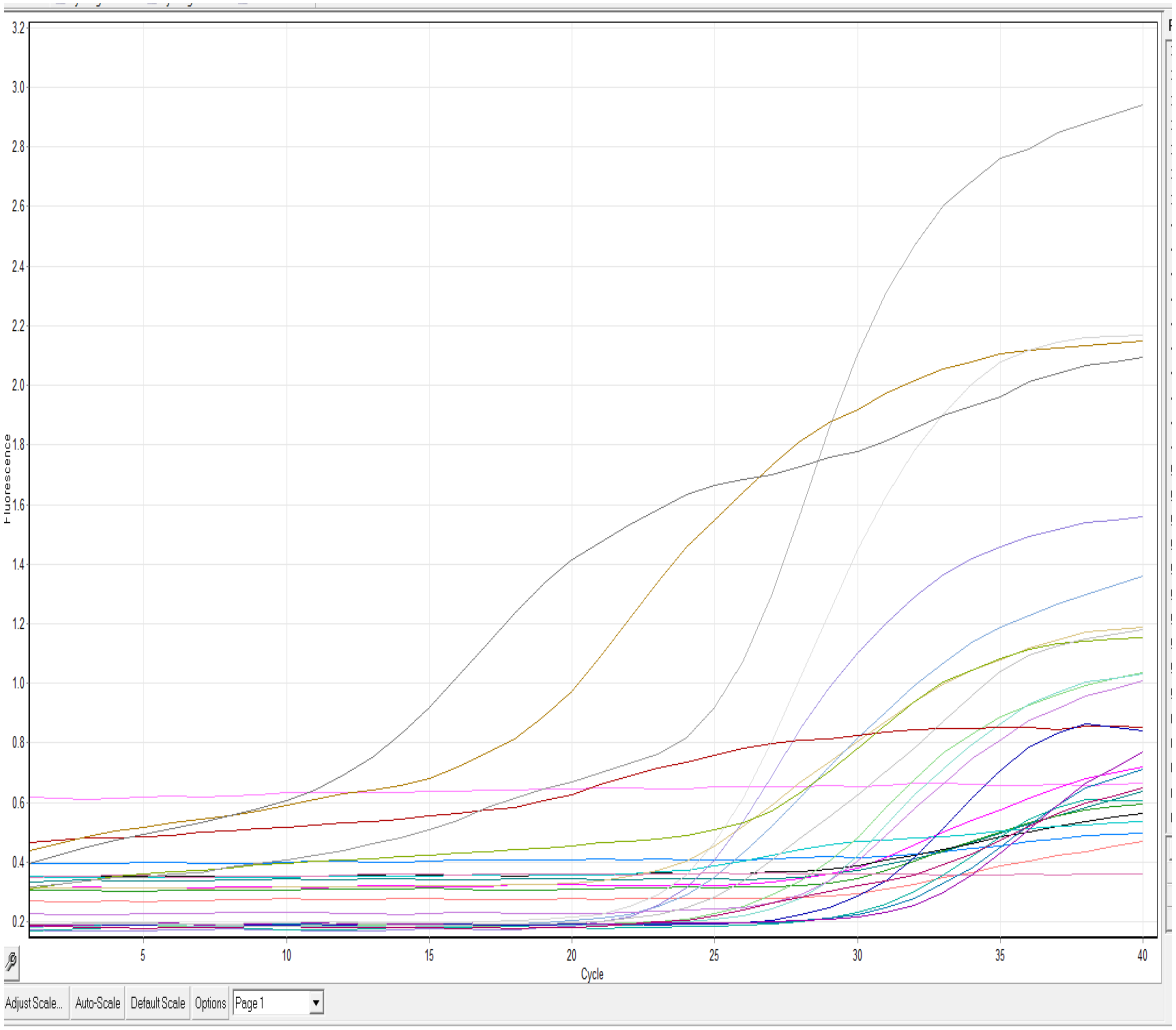
Fig. 1. Real-time LightCycler PCR assay on four *Leishmania* species using primers JW11 and JW12 (A and C) or primers JW13 and JW14 (B and D) amplifying a DNA fragment from the kinetoplast DNA. Gel electrophoresis of PCR products was done (A and B) as well as melting curve analysis (C and D). (A and B) Lanes 1, 2, 3 and 4 represent PCR products obtained from *L. infantum* 2385, *L. major* NIH173, *L. donovani* LV9 and *L. tropica* strain Vehda, respectively.

Summary of the results



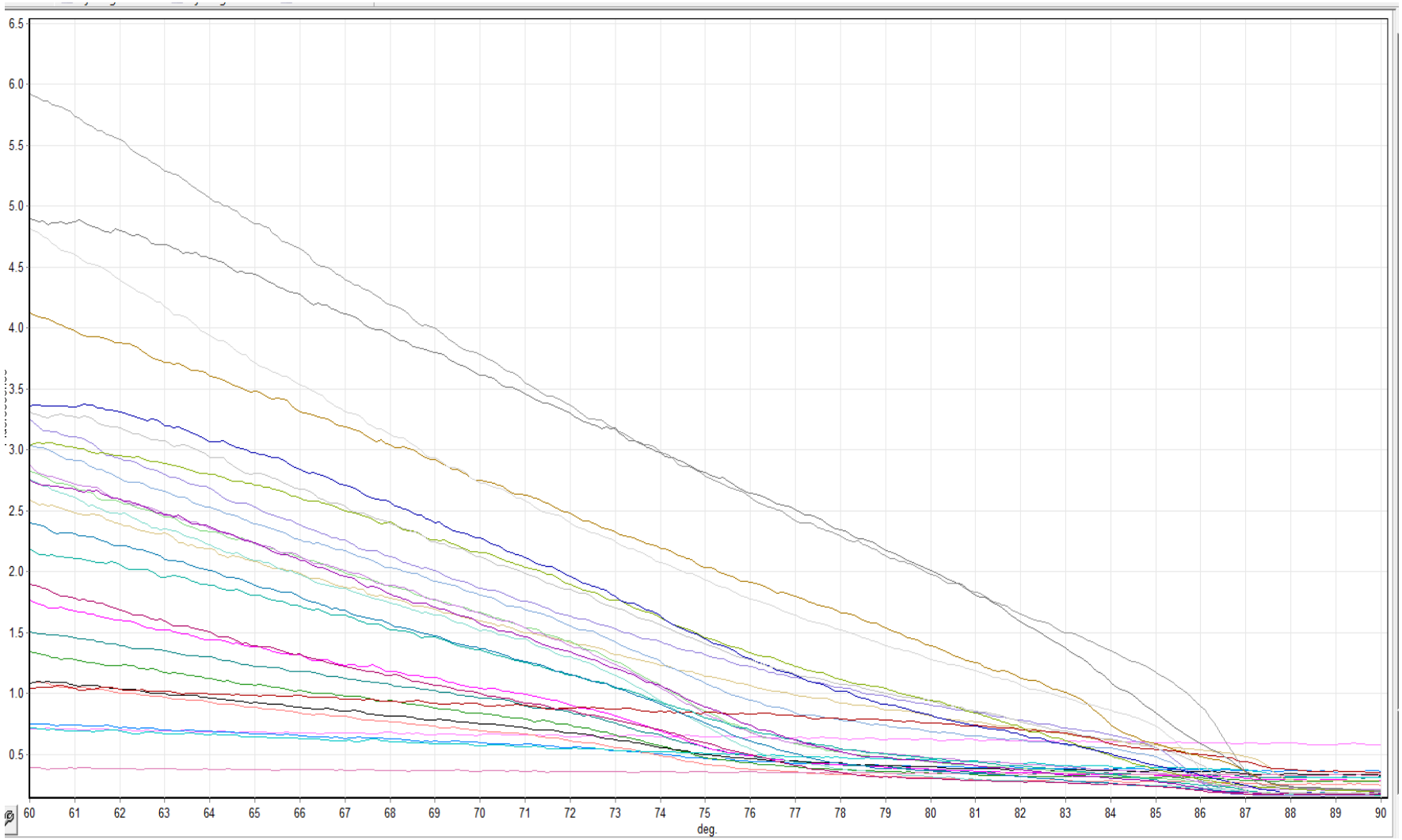
	Blood	Skin Biopsy	Sand fly	Positive Controls	Total
Total Examined	21	14	14	14	63
Number Positive	11	9	6	12	38
Proportion (%)	52.4	64.3	42.9	85.7	60.3

Summary for qPCR



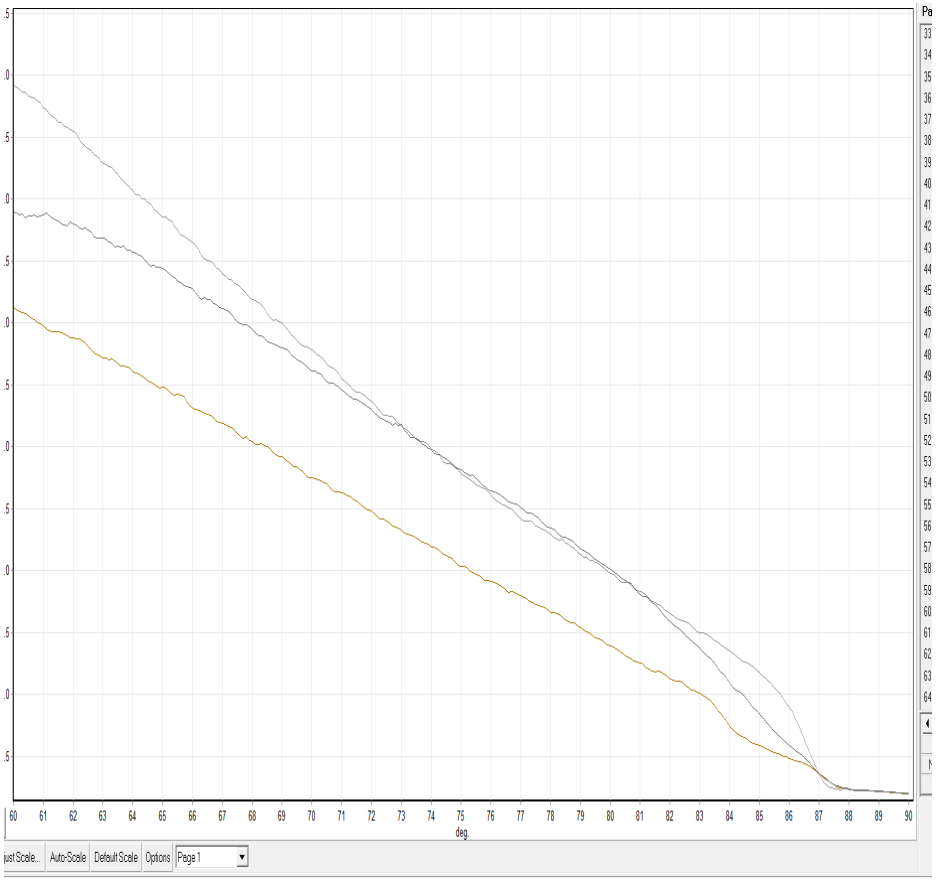
- Low florescence

HRM curves


6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90
deg.

HRM

Experimental Comparison



Ideal

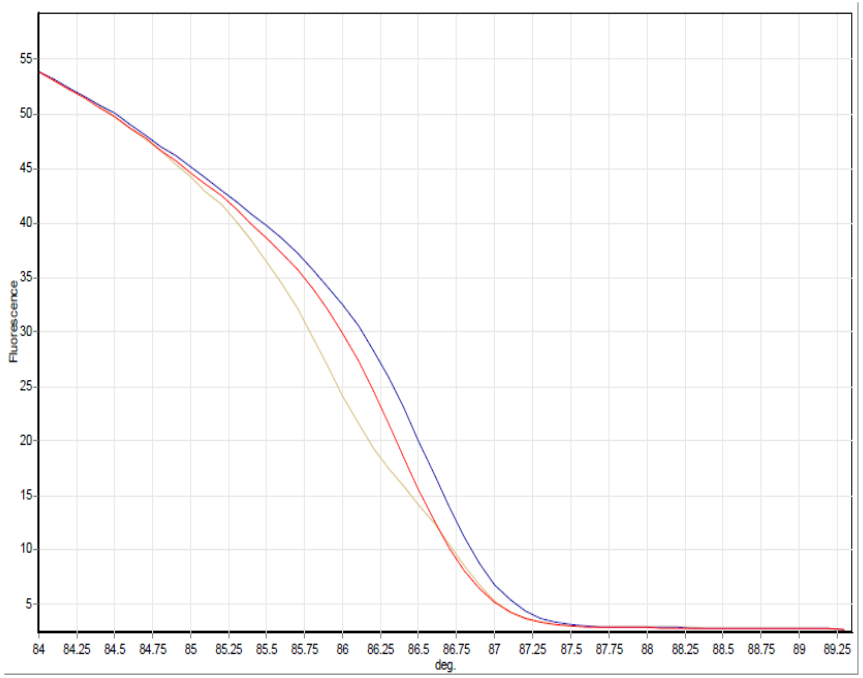
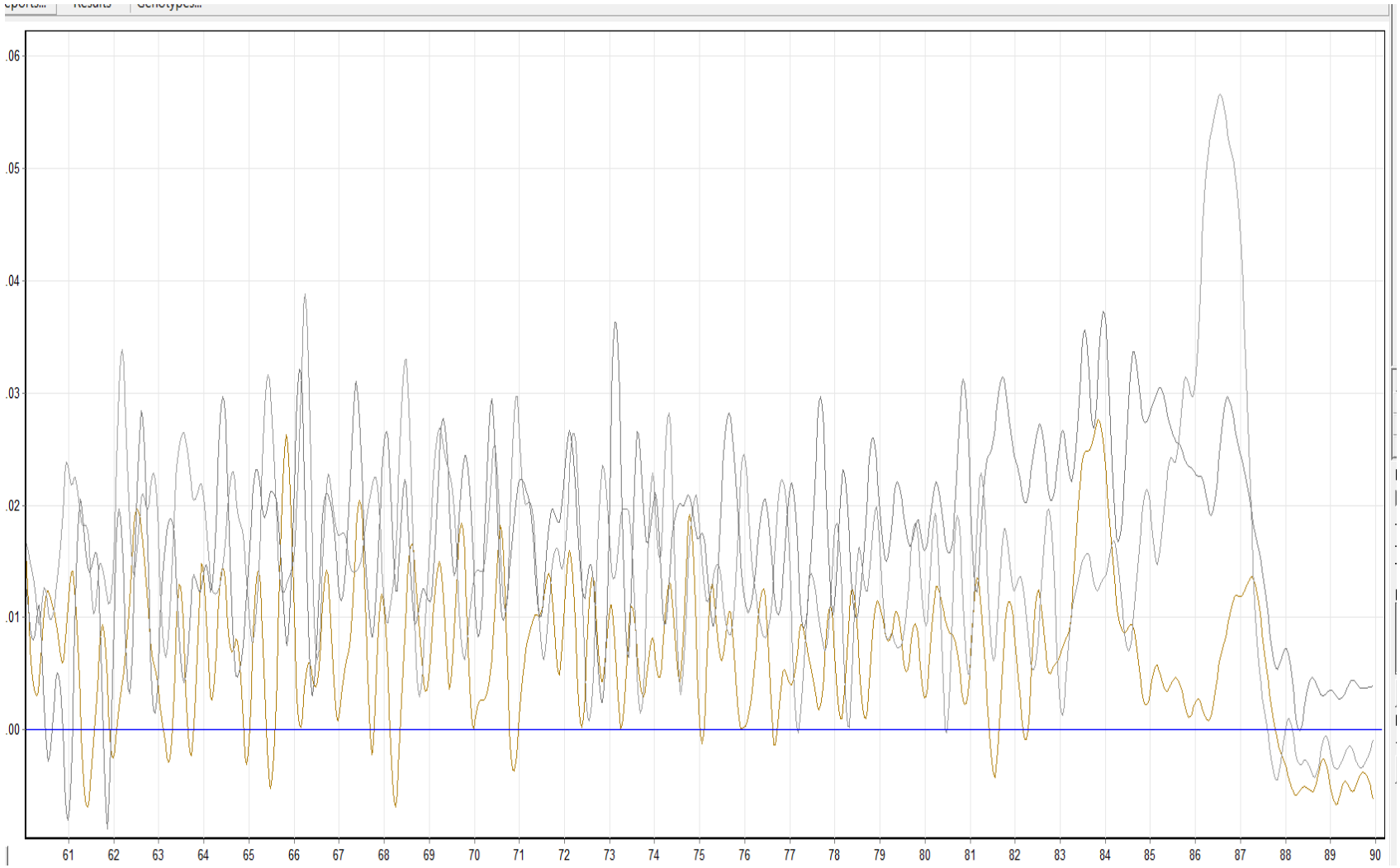


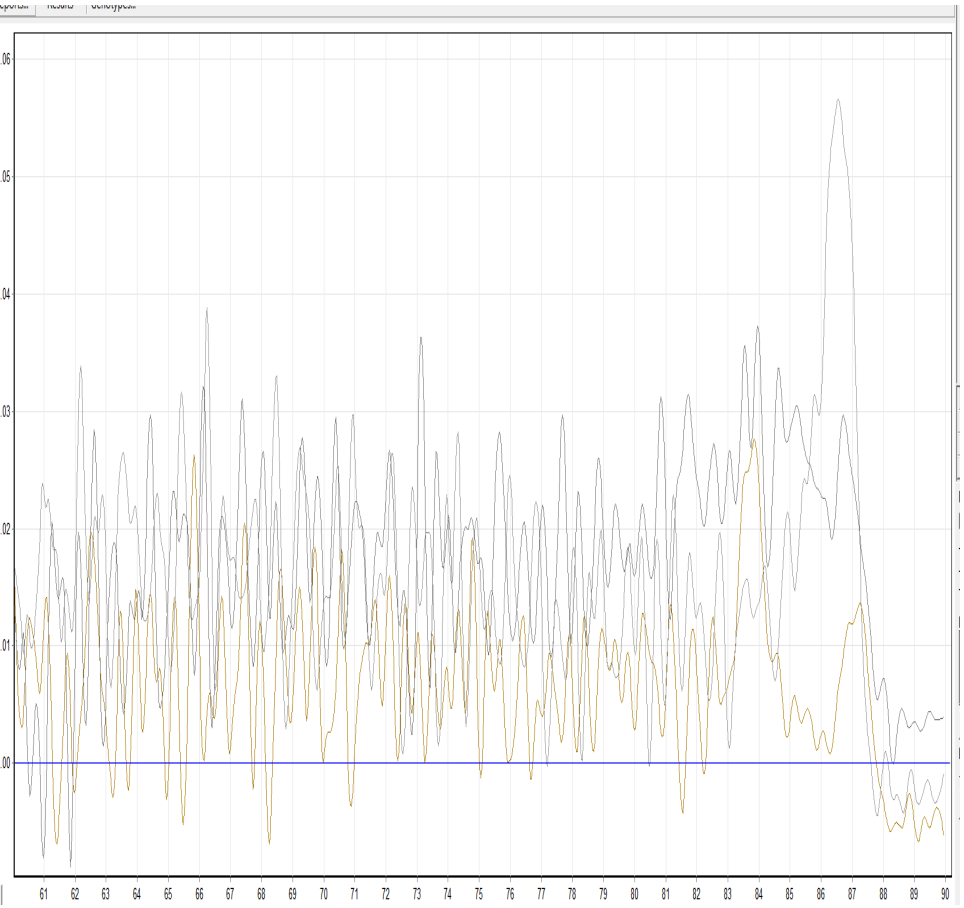
Figure 3. Example of Class 4 SNP genotyping by HRM on the Rotor-Gene 6000; discrimination of monocarboxylate transporter 1(MCT1; A1470T) alleles. *MCT1 is a representative class 4 SNP (A to T conversion)—the rarest and most difficult SNP type to discriminate. HRM analysis identified a characteristically subtle change in T_M of only about 0.2°C between homozygous samples. The melt profiles shown are; AA homozygote, blue (right); TT homozygote, red (middle) and the AT heterozygote, yellow (left) . Heteroduplexes are discriminated by a change in the shape of the*

HRM Melting for Experiments / Comparison

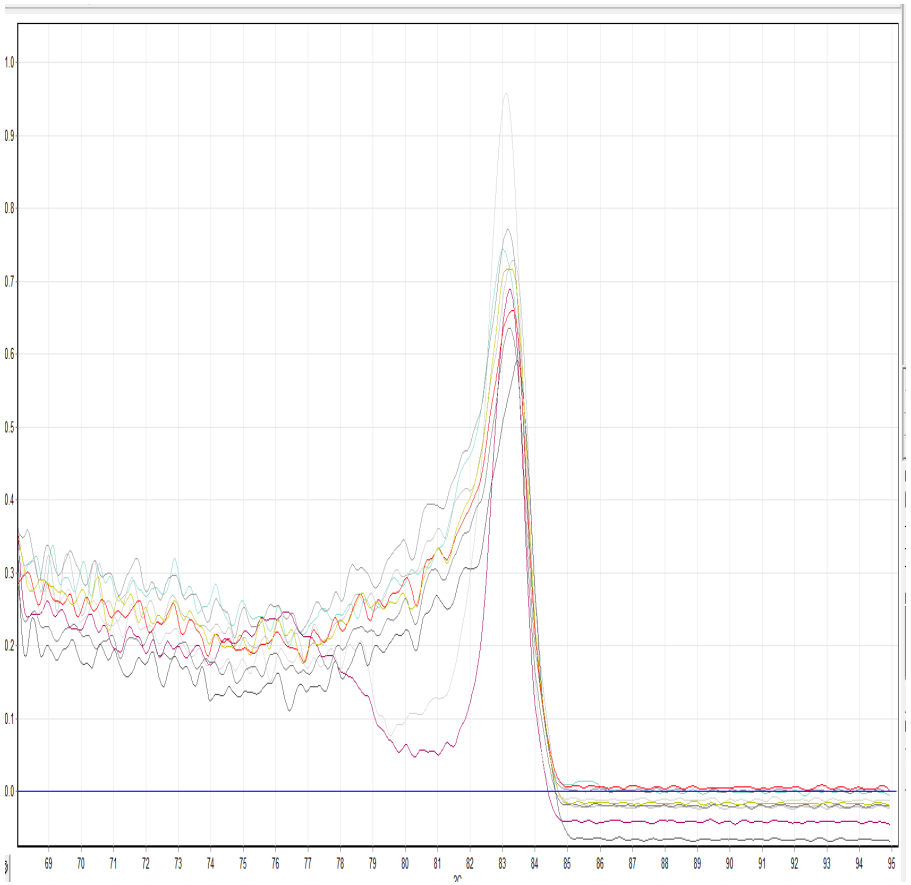


HRM

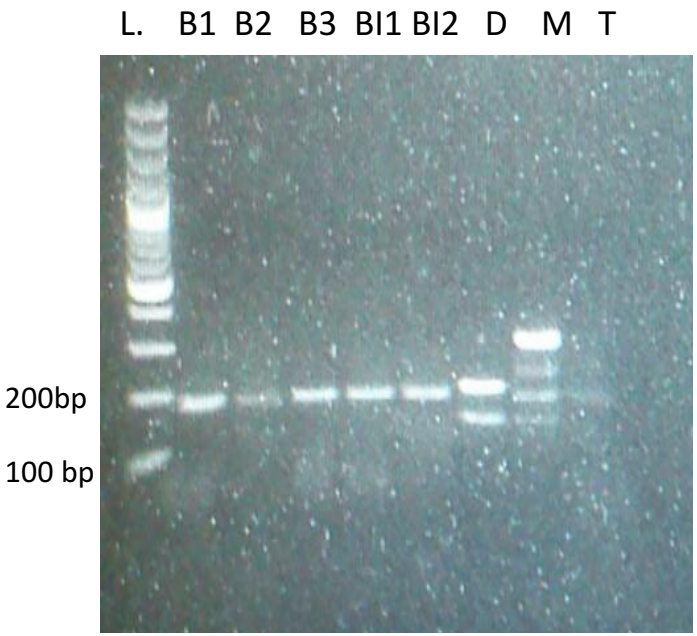
Experimental Comparison



Ideal



RLFP assay



L= DNA ladder
B= blood sample
BI= Biopsy sample
T= *L.tropica*
M= *l.major*
D= *l.donavani*

Advantages/Disadvantages

- HRM can differentiate between species
- High sensitivity /kDNA ~10,000 copy
- Allow the identification of non specific products
- No Gel

- Longer sequence to amplify ~600 bp
- Low efficiency
- 1-10 parasites
- Sybr green binds to double strand (Primer dimers)

Other techniques

- Multiplex qPCR
- Nested qPCR
- Sequencing
- Multi-locus sequence typing (MLST) 4-6 house keeping genes

Conclusion

- Amplification of 600 bp had a lower efficiency
- Lower sensitivity with false Negative
- RFLP amplified a smaller sequence increasing the efficiency of the assay and restriction enzyme allowed the identification of the species
- other molecular technique can be more sensitive