HIGH RESOLUTION MELTING ANALYSIS (HRM) FOR *LEISHMANIA* SPECIES IDENTIFICATION

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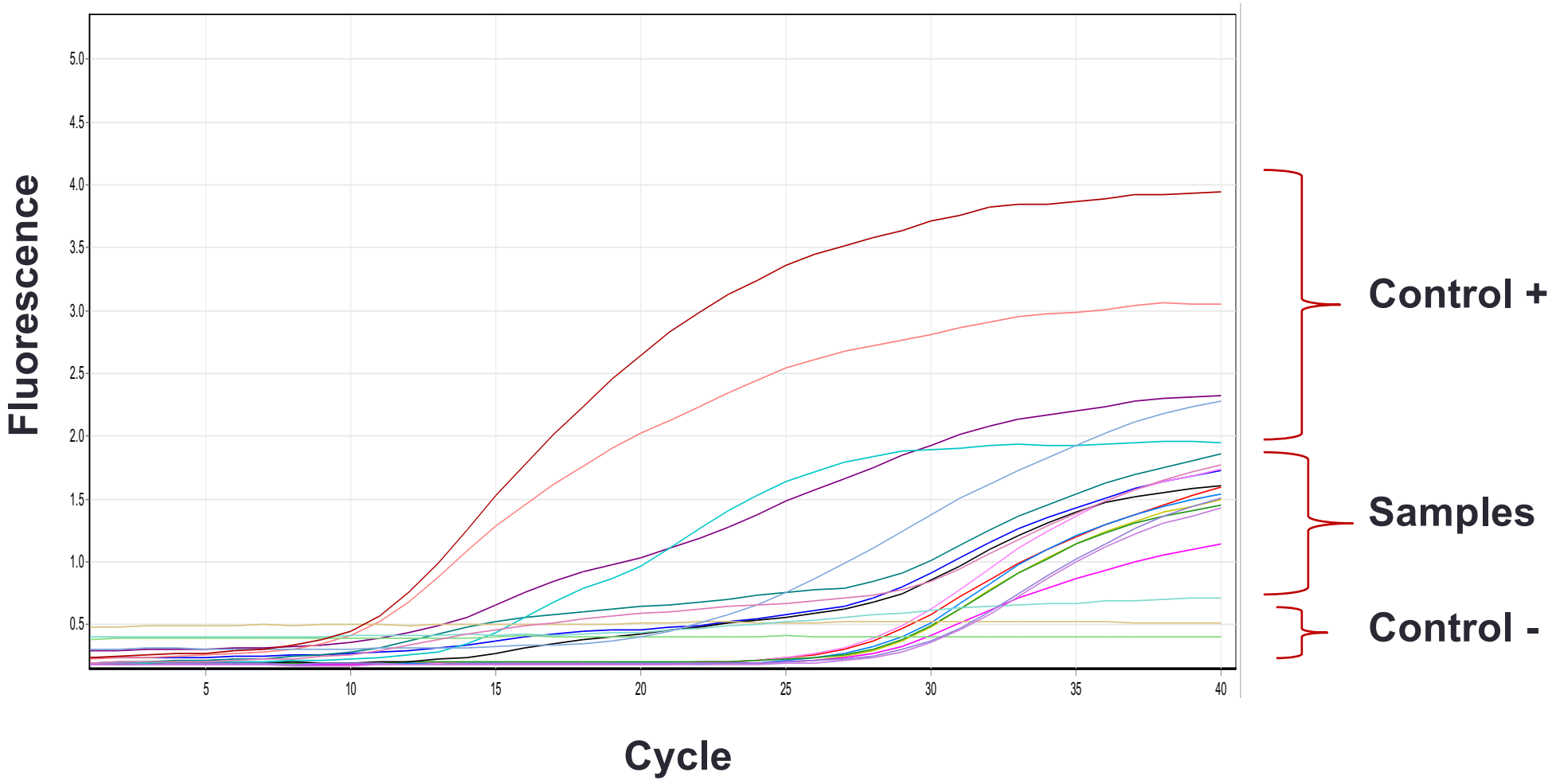
High Resolution Melting (HRM) analysis

- New post-PCR analysis method used to identify variations in nucleic acid sequences.
- Detection of small differences in PCR melting curves.
- It is enabled by improved dsDNA-binding dyes used in conjunction with real-time PCR instrumentation.

qPCR Results for DNA amplification of Blood samples

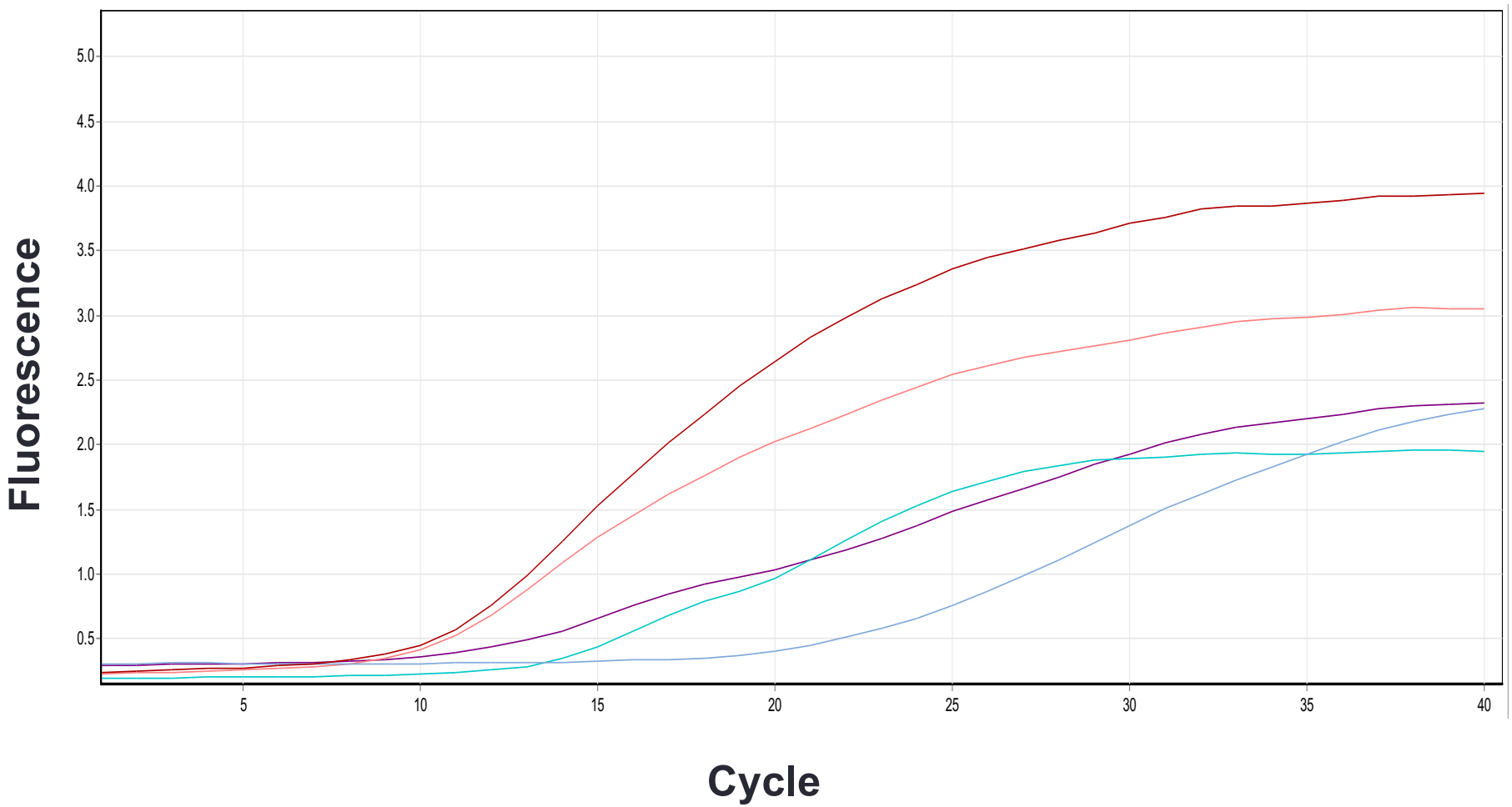
Real-time LightCycler PCR assay for 20 samples using primers JW13 and JW14 amplifying a DNA fragment from the kinetoplast DNA

(Nicolas et al. 2002)

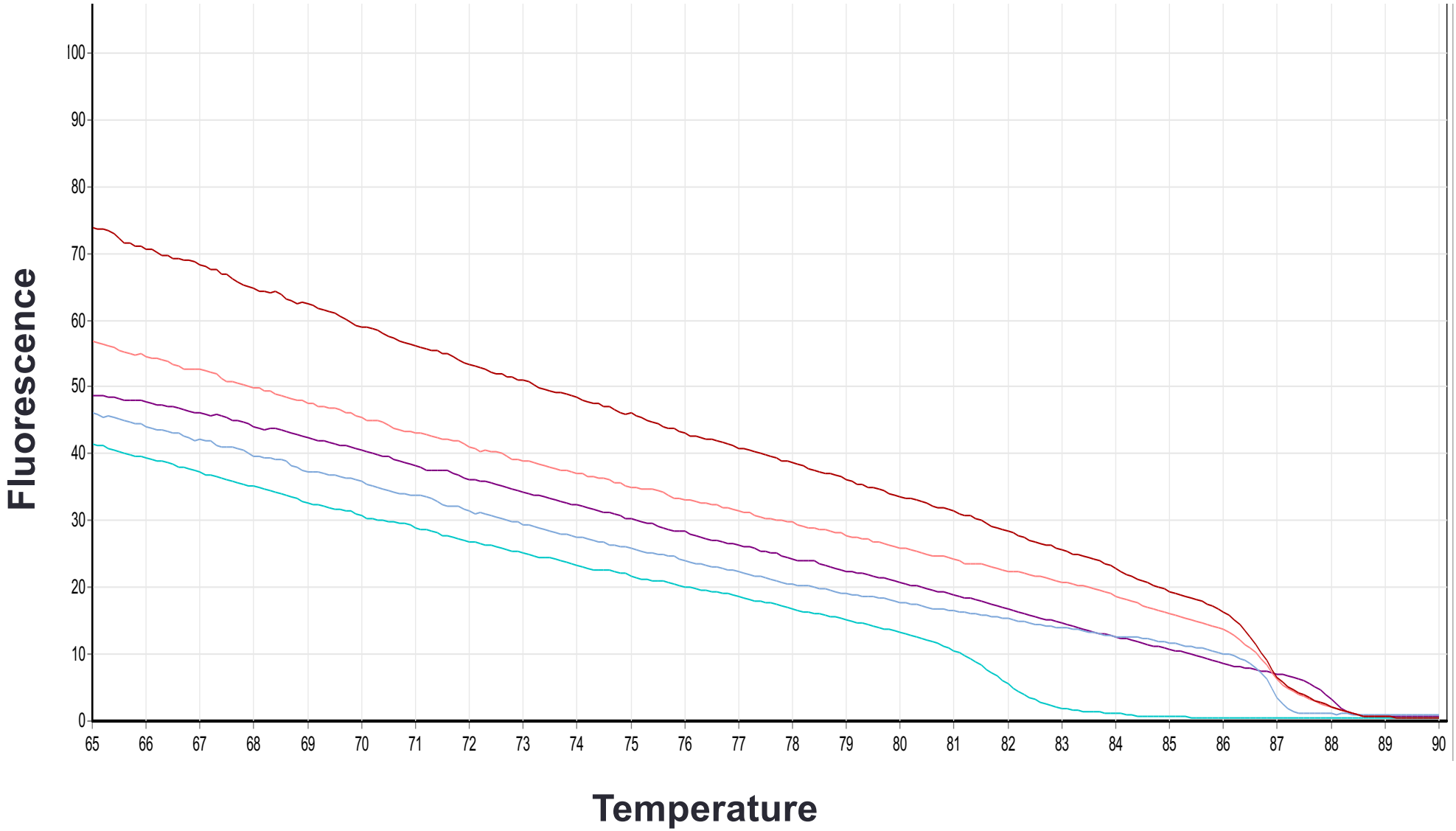


qPCR Results for DNA amplification of Blood samples

Control +



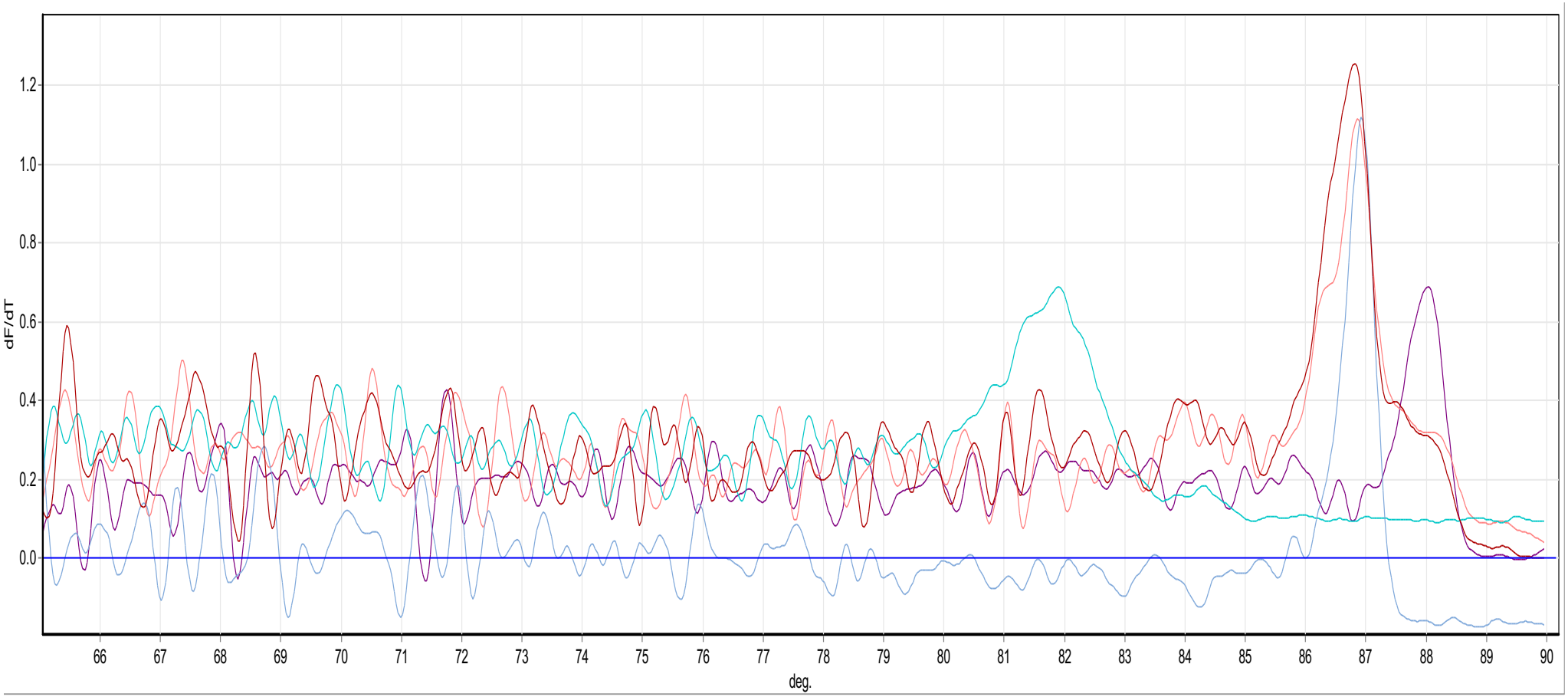
Data acquisition



Melting curve analysis for species identification

Real-time LightCycler PCR assay using primers JW13 and JW14

amplifying a DNA fragment from the kinetoplast DNA

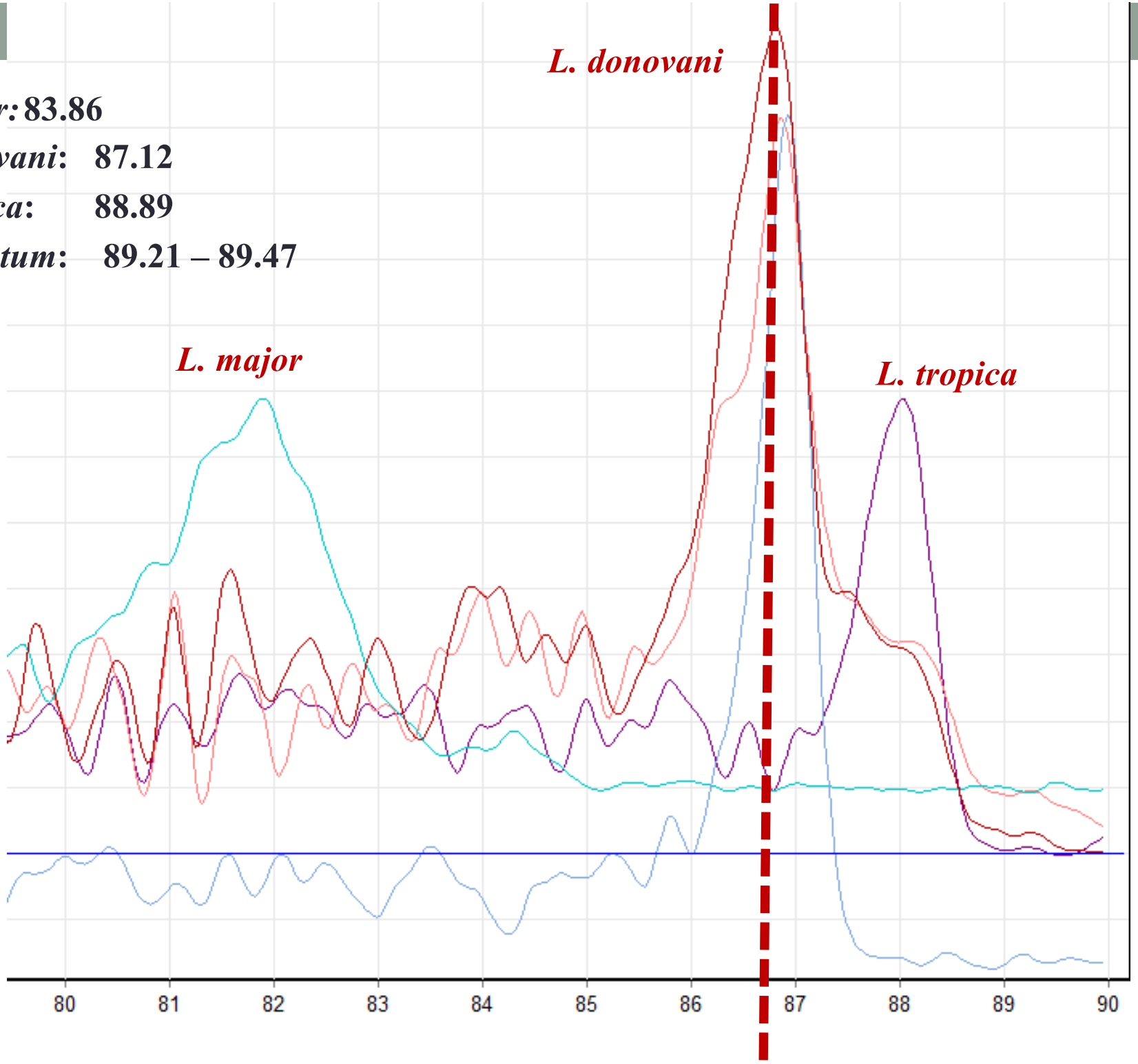


L. major: 83.86

L. donovani: 87.12

L. tropica: 88.89

L. infantum: 89.21 – 89.47



Advantages of HRM

- **Low reagent consumption**
  - (requires only PCR reaction volume (20 µL) for analysis of each sample).
- **Simple, fast workflow:**
  - no additional instrumentation is required after PCR amplification.
- **Low sample consumption:**
  - Following HRM analysis, the PCR amplicon can be used directly in a Sanger sequencing reaction

Disadvantages of HRM

- **Limited sensitivity.**
- **Limited to genes characterized by low rate of mutations**
- **Returning to others methods in case of co-infection.**