TARGETING THE SPLICED LEADER RNA:
ACCURATE DIAGNOSIS OF HUMAN AFRICAN TRYPANOSOMIASIS

Stijn Deborggraeve, Pablo Andrade Gonzalez, Philippe Büscher

Parasite Diagnostics Unit
Institute of Tropical Medicine Antwerp
Belgium
1. THE SPLICED LEADER RNA

- *Trypanozoon* specific RNA sequence of 39 bp
- Chimera with all mRNA in the cell
- mRNA is considered to be the best marker for viable parasites

Would the SL RNA be a good target for molecular diagnostics?

2. METHODOLOGY

- **SL-RNA**
- **mRNA**

**REVERSE TRANSCRIPTION**

**SL-RNA cDNA (39 bp)**

**DETECTION BY QUANTITATIVE REAL-TIME PCR**
3. PROOF-OF-CONCEPT USING SYNTHETIC SPLICED LEADER RNA

**Melting Peaks**

- 100 ng synthetic SL-RNA
- neg control
- cDNA prep
- neg control
- PCR

**Temperature (°C)**

45 50 55 60 65 70 75 80 85 90 95

**didT Fluorescence (485-510)**

4. DETECTION LIMIT ON SYNTHETIC SPliced LEADER RNA

Melting Peaks

- 0.005 ng
- 0.05 ng
- 0.5 pg
- 0.5 ng
- 0.05 pg
- 0.005 fg
- 0.5 fg
- 0.05 fg
- Dilutions from 0.005 fg to 0.0005 fg
5. SL-RNA DETECTION IN SERIAL DILUTION OF *T.b. gambiense* RNA

![Graph showing melting peaks for different dilutions of T.b. gambiense RNA](image)

260 ng to 260 fg *T.b. gambiense* RNA
5. SPLICED LEADER RNA DETECTION IN SERIAL DILUTION OF *T.b. gambiense* RNA
6. SL-RNA DETECTION IN SERIAL DILUTIONS OF *T. b. gambiense* PARASITES IN BLOOD

![Graph showing melting peaks for E6 to E4 p/mL, E3 p/mL, E2 p/mL, E1 p/mL, and human blood control.](image-url)
7. ANALYTICAL SPECIFICITY

- Melting Peaks

- Temperature (°C)

- Fluorescence (465-510)

- T. b. gambiense
- T. b. rhodesiense
- T. b. brucei
- T. evansi
- T. equiperdum
- T. vivax
- L. donovani
- T. congolense
- T. rangeli
8. HOW MANY SL-RNA MOLECULES ARE THERE IN ONE SINGLE PARASITE?

2,000,000 p

> 9000 SL-RNA molecules/cell

The standard curve of synthetic SL-RNA molecules.
9. CONCLUSIONS & PERSPECTIVES

- WE HAVE EXPLORED THE PARASITE’S MESSENGER RNA AS A DIAGNOSTIC TARGET

- THE SL-RNA HAS GREAT POTENTIAL AS A TARGET FOR MOLECULAR DIAGNOSTICS

- THE LIMIT OF DETECTION IS 100 PARASITES / ML OF BLOOD

- TRYPANOZOOON SPECIFIC DETECTION IS POSSIBLE

- THE SL-RNA COPY NUMBER PER CELL IS AT > 9000

- LABORATORY EVALUATION STUDY IN RNA FROM PATIENTS AND CONTROLS IS ONGOING

- TRANSLATION INTO A USER-FRIENDLY DIAGNOSTIC FORMAT
10. ACKNOWLEDGEMENTS

Pablo Andrade Gonzalez
Philippe Büscher
Nicolas Bebronne
Bruno Bucheton
Jose Ramon Franco
Research Foundation Flanders