Development of two promising alternatives for animal trypanosomosis diagnosis:

- molecular detection of 7SL-derived small RNA
- microsphere-based immunoassay (Luminex®)

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Trypanozoon associated diseases

- **Equine trypanosomosis** comprises 3 diseases caused by protozoa of the subgenus *Trypanozoon*: *Trypanosoma equiperdum* (dourine), *T. evansi* (surra), and *T. brucei* (nagana) **(Table 1 and Fig. 1)**. These diseases are notifiable to the World Organisation for Animal Health (*WOAH, previously OIE*). Certificates of negative serological test are required for international trade and movement of equids. Due to the absence of vaccine and recurring treatment failure, **the development of sensitive and specific diagnostic tests remains crucial for controlling these diseases.**

Table 1. *Trypanozoon associated animal diseases*

Diseases	Sub-species	Main transmission routes	Host range
Nagana	*T. brucei brucei*	 Tsetse fly	Multi-species
Surra	*T. brucei evansi*	 Stomoxys  Tabanus	Multi-species
Dourine	*T. brucei equiperdum*	 Mating	Equids

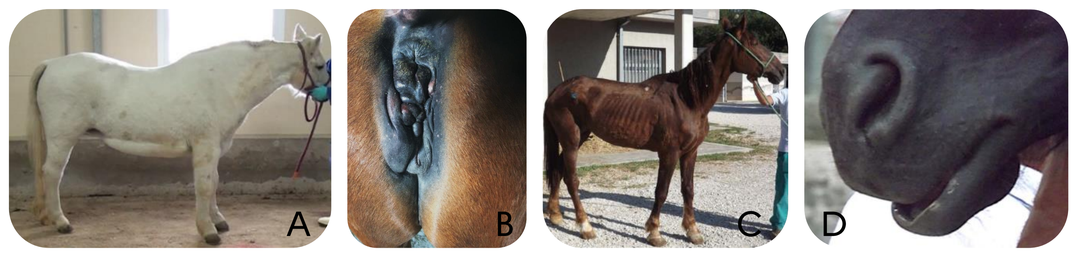
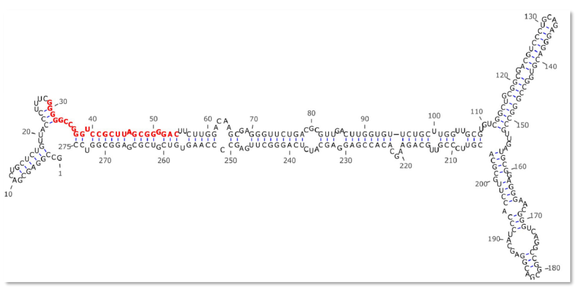


Figure 1. *Clinical signs observed including (A) ventral edema (B) genital edema, (C) weight loss and (D) labial ptosis.*

Molecular detection of 7SL-derived small RNA

- **7SL-derived small RNA** is a 26 nt RNA sequence present at high levels in the blood animals infected by trypanosomes, identified as potentially useful diagnostic biomarker detectable using a two-step RT-qPCR in the **Roslin Institute**[1] **(Fig. 2)**.



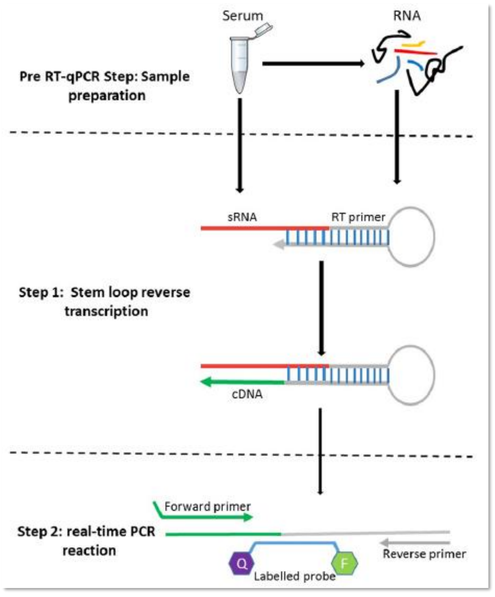


Figure 2. *(A) Predicted secondary structure of the* T. congolense *7SL RNA. The 7SL-sRNA is highlighted in red, (B) Schematic representation of the Taqman RT-qPCR assay, a 2 step based assay that makes use of stem loop primers*[1].

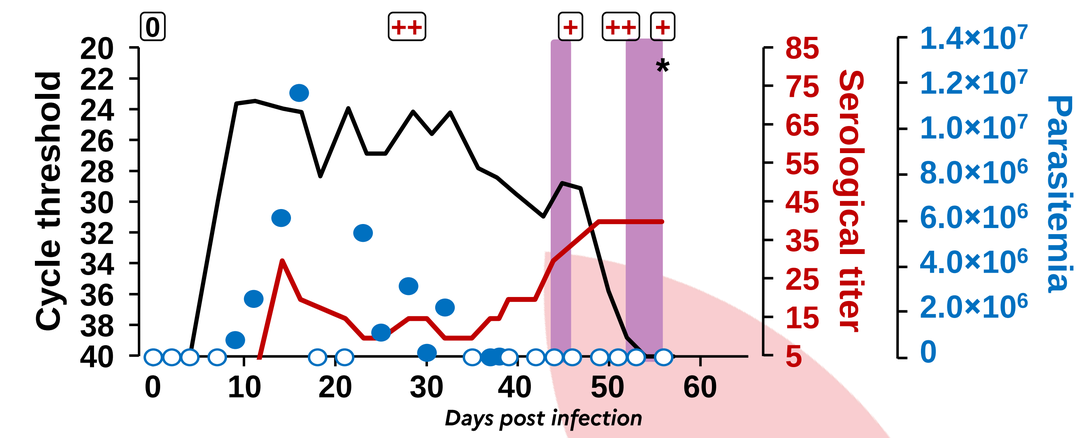


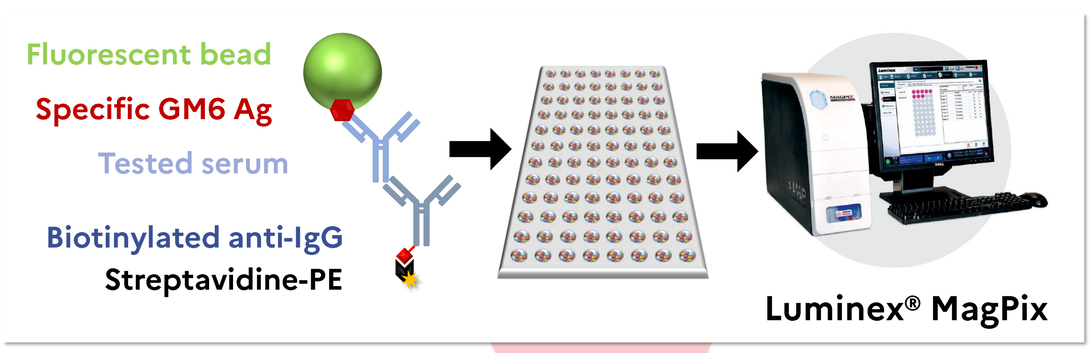
Figure 3. *In vivo* detection of *T. equiperdum* specific 7SL-sRNA. *Melarsomine treatment (0.5 mg kg⁻¹ day⁻¹) is represented by purple areas. Asterisk indicates the end point of the experiment.*

- 6 horses were experimentally infected with *T. equiperdum*. The 7SL-sRNA signal was detected between 2 and 7 days after infections. The signal remained detectable during subpatent parasitemia **(Fig. 3)**.
- 7SL-sRNA remain stable in positive sera 7 days at either 4°C, room temperature, or 30°C.

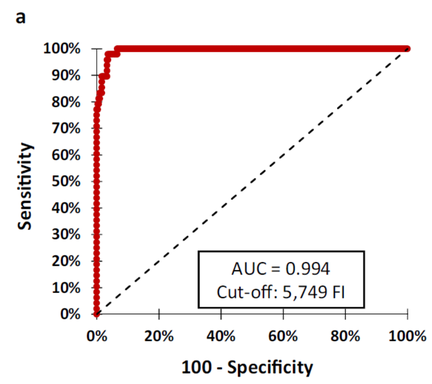
7SL-sRNA detection combines the sensitivity of serology methods with specificity of molecular detection[2]

xMAP® (Luminex®) immunoassay

- A microsphere immunoassay based on the xMAP® technology (Luminex) was performed.



- 8 recombinant antigens were evaluated (enolase, GM6, PFR1, PFR2, ISG65, VSGat).



b

	Positive sera (+)	Negative sera (-)
Luminex (+)	47	12
Luminex (-)	1	289

c

	Values	95 % CI
Sensitivity	97.9 %	87.9 % - 100 %
Specificity	96.0 %	93.1 % - 97.8 %
+ LR	25.56	14.09-42.82
- LR	0.02	0.0-0.15

Figure 3. *Sensitivity and specificity obtained with the GM6 antigen (a) ROC analysis using 301 negative and 48 positive sera. (b) Contingency analysis of the 349 sera. (c) Sensitivity, specificity values and Likelihood ratio (LR).*

- The GM6 antigen showed the best performance with a sensitivity of 97.9% and a specificity of 96.0% (Fig. 4).

Luminex GM6 constitutes a new serological diagnosis of equine trypanosomosis[3]

The performance of the 7SL-sRNA detection and the Luminex GM6 needs to be confirmed on a larger number of field samples but these technics already constitute promising methods for the diagnosis of equine and animal trypanosomosis

1. **Chiweshe SM, Steketee PC, Jayaraman S, Paxton E, Neophytou K, et al.** (2019) Parasite specific 7SL-derived small RNA is an effective target for diagnosis of active trypanosomiasis infection. PLOS Neglected Tropical Diseases 13(2): e0007189. https://doi.org/10.1371/journal.pntd.0007189.
2. **Verney M., Grey F., Lemans C., Géraud T., Berthier D., Thévenon S., Rincé A., Hans A., Morrison L., Hébert L. (2020).** Molecular detection of 7SL-derived small RNA is a promising alternative for trypanosomosis diagnosis. TransboundEmerg Dis.; 67:3061–3068. https://doi.org/10.1111/tbed.13744
3. **Verney M., Gautron M., Lemans C., Rincé A., Hans A., Hébert L.**, Development of a microsphere-based immunoassay for the serological diagnosis of equine trypanosomosis. Scientific Reports 12 (1): 1308. https://doi.org/10.1038/s41598-022-05356-y.

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