anses

National reference laboratory for **DOURINE**

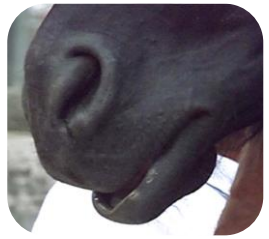
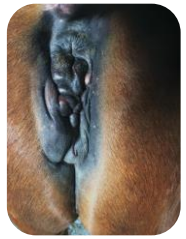
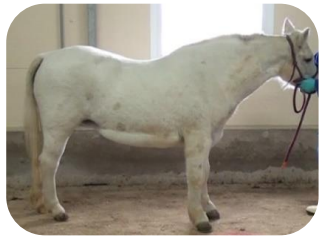
European Union Reference Laboratory for EQUINE DISEASES

World Organisation for Animal Health
Founded as OIE

WOAH Reference Laboratory for DOURINE

DEVELOPMENT OF TWO ALTERNATIVES FOR TRYPANOSOMOSIS DIAGNOSIS:

- THE MOLECULAR DETECTION OF 7SL-DERIVED SMALL RNA &
- A MICROSPHERE-BASED IMMUNOASSAY (Luminex®)

ANSES, Laboratory for Animal Health, Normandy site, FRANCE
Physiopathology and Epidemiology of Equine Diseases unit (PhEED)
Parasitology team


Mylène Verney


Morgane Gautron


Charlène Lemans


Laurent Hébert

Objectives

- Develop sensitive and specific diagnostic tools
- For first-line or confirmatory diagnosis
- In the ideal: field application

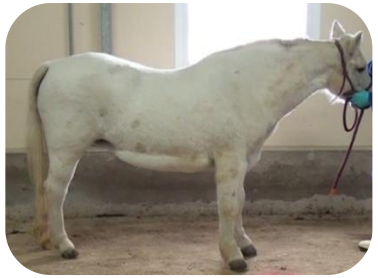
Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Detection of the agent[1]						
Microscopic observation	–	+	+	+++	–	–
PCR/ real-time PCR	–	+	+	+++	+	–
Detection of immune response						
CFT	++	+++	+++	+++	+++	–
IFAT	++	+	++	+	++	–
ELISA	+++	+	+++	+	+++	–
ICT	+	+	+	+	+	–

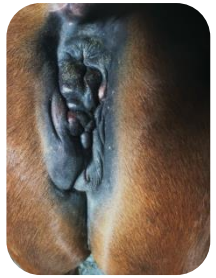
7SL-sRNA detection

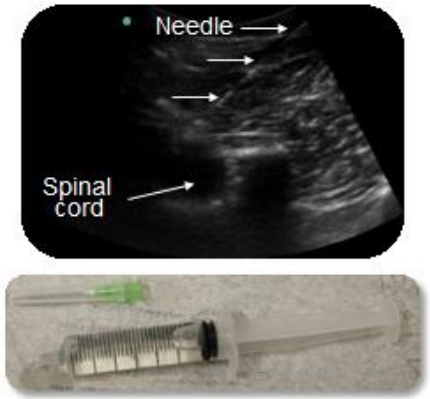
Microsphere-based immunoassay (Luminex®)

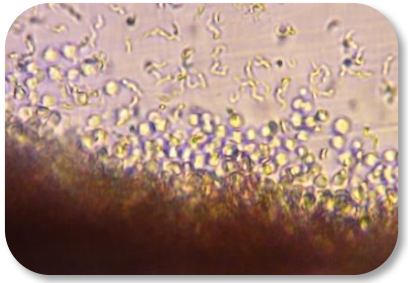
Origine of positives sera

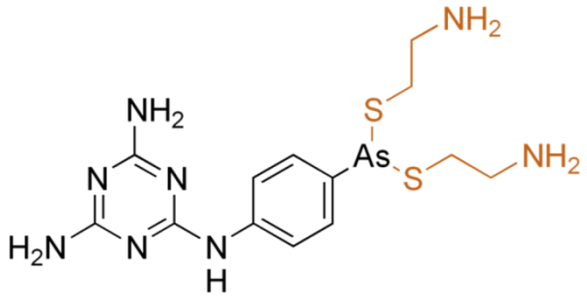
- **Horses expérimentally infected with *T. equiperdum OVI***



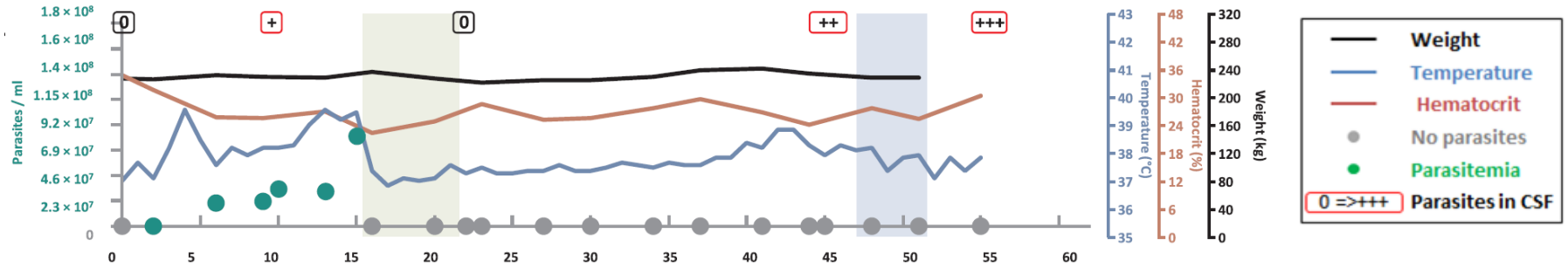








Melarsen oxide + 2 Cysteamine



Treatment failure

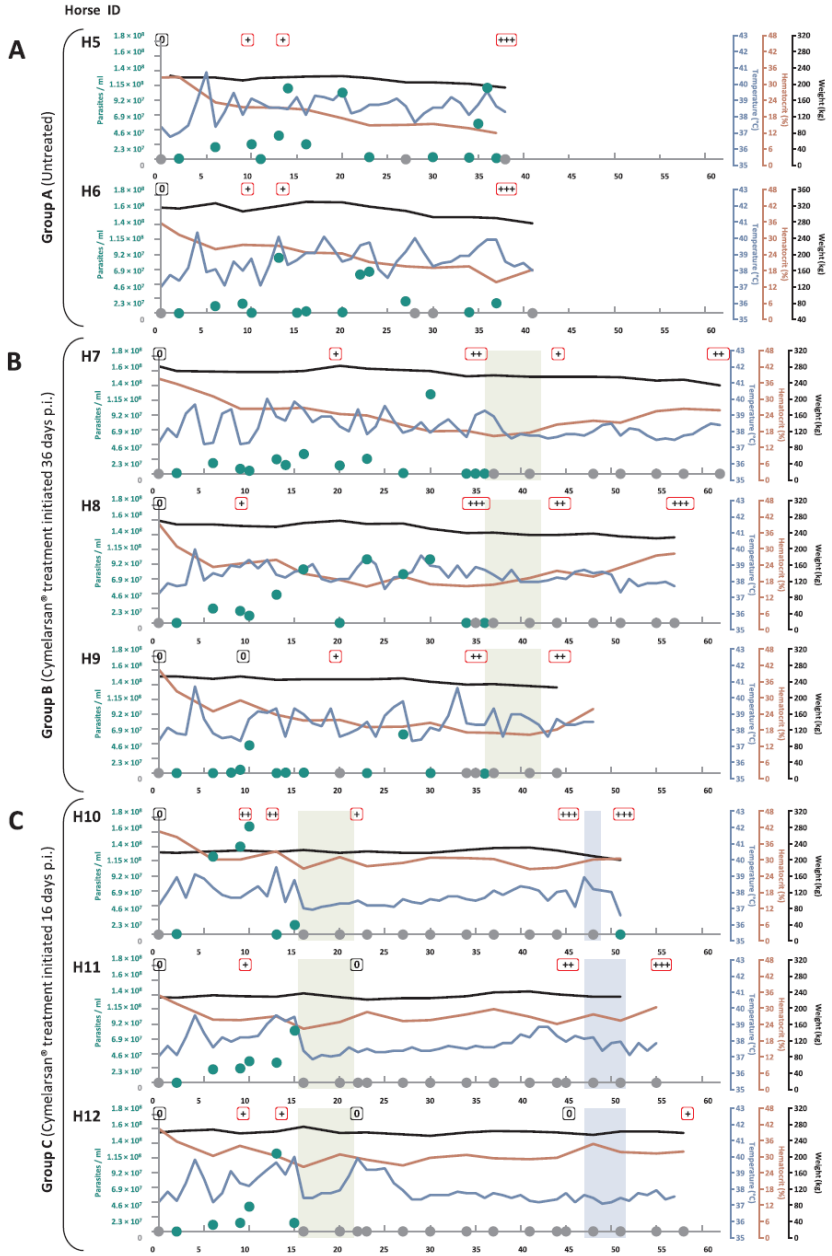
(Hébert et al., 2018a and 2018b)

Origine of positives sera

- Sampled from the 12 pony mares
- 36 sera from endemic countries:
  - Argentina (n = 34)
  - Italy (n = 1)
  - Mongolia (n = 1)

Origine of negative sera (±300)

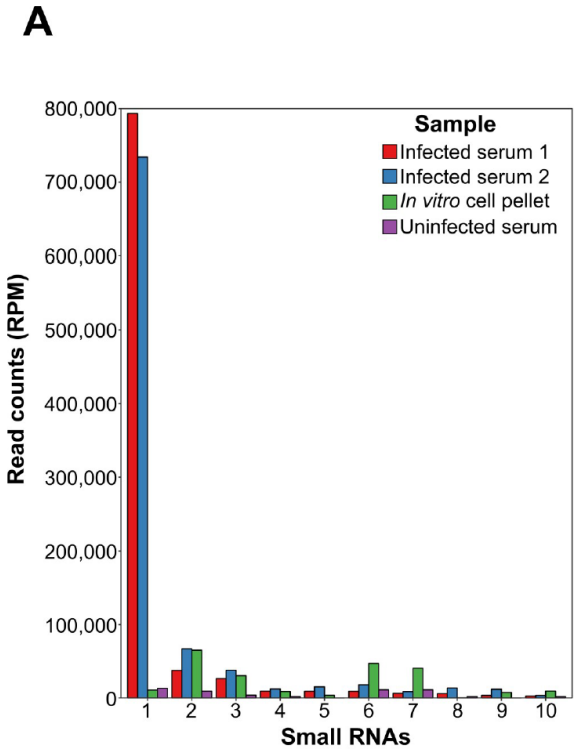
- 164 sera collected between 2015 and 2017 in France
- 99 from an equine piroplasmosis study
- 13 were positives for other equine diseases
- 20 sera were sampled before experimental infection

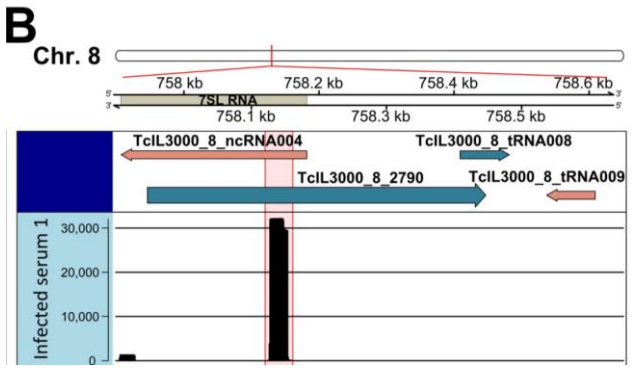


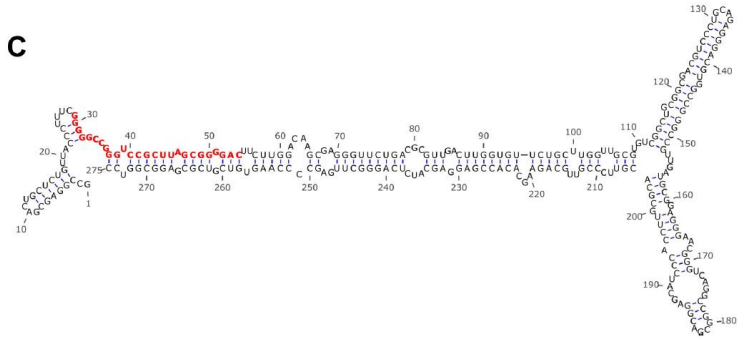
(Hébert et al., 2018a and 2018b)

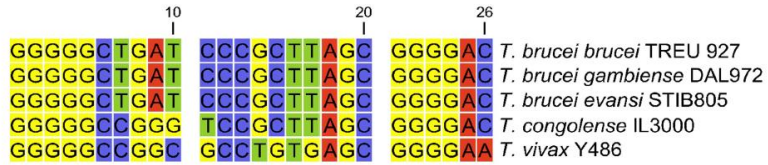
7SL-sRNA as a potential diagnostic biomarker

- RNA-sequencing of serum isolated from *T. congolense*-infected cattle









An abondant **secreted** small RNA

Small RNA derived from 7SL gene: 7SL-sRNA **(26 nt)**

Sequence with species-specific differences

(Chiweshe et al, 2019)

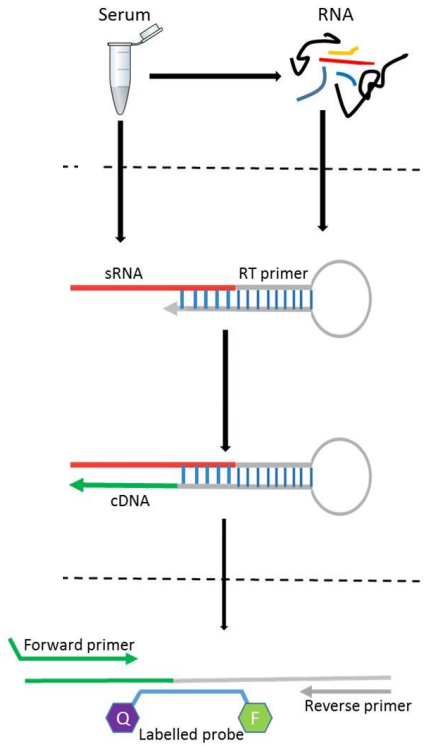
7SL-sRNA constitutes a promising target

- Amplification of the 26 nt by a double step RT-PCR

1- **RNA Extraction**
TRIzol™
Chloroform
Isopropanol

2- **Reverse transcription**
Stem loop RT primer

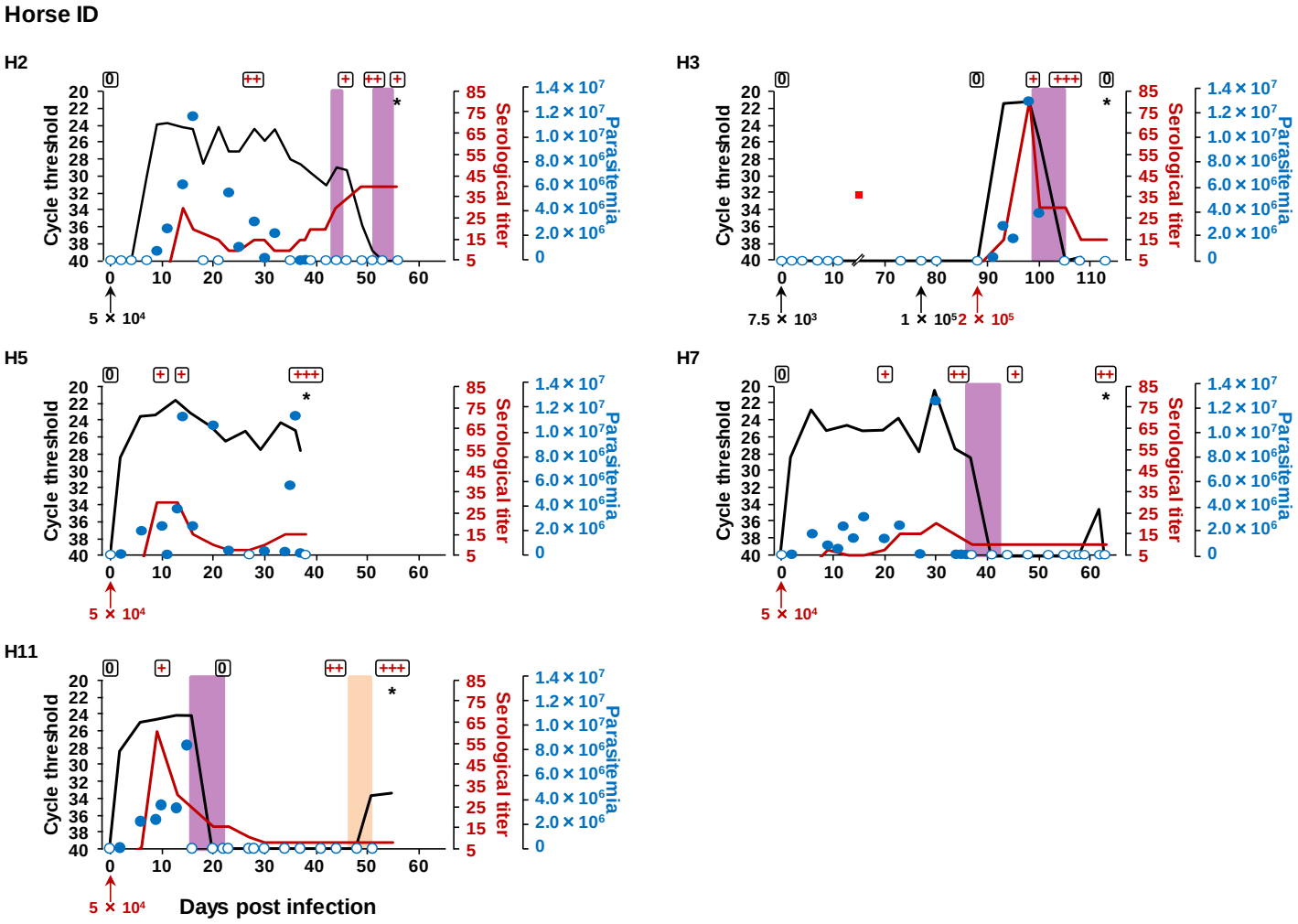
3- **Real Time PCR**
TaqMan®



Aims: determine whether 7SL-sRNA could constitute a suitable biomarker for diagnosis of *Trypanozoon* infection in equids.

(Chiweshe et al, 2019)

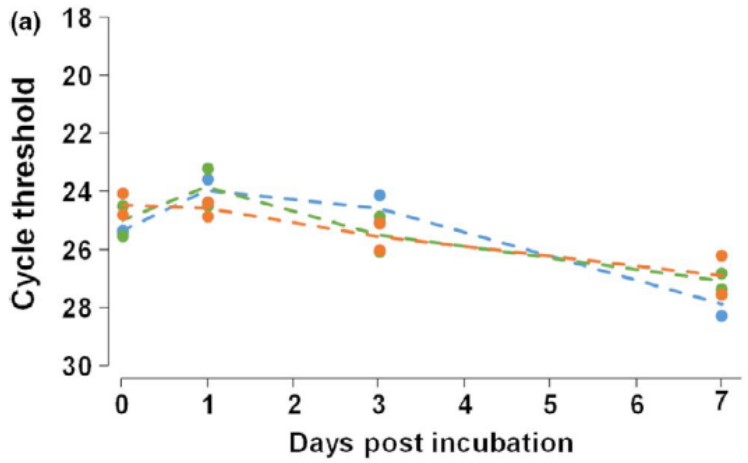
7SL-sRNA detection in horses infected by *T. equiperdum*



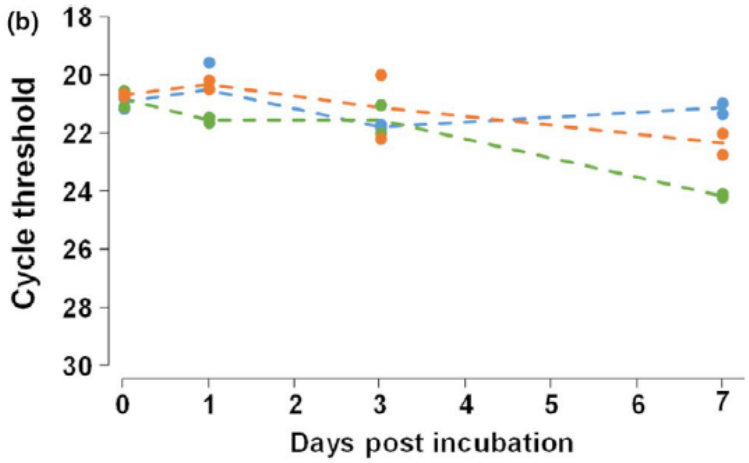
- **Rapid accumulation of 7SL-sRNA** in blood after infection
- Trypanocide treatment (Cymelarsan®) induces a rapid disappearance of the 7SL-sRNA signal
- Allow the **early detection of parasite relapse**

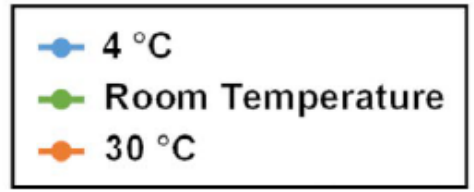
Stability of 7SL-sRNA in equine sera

Medium positive serum



Strongly positive serum





The 7SL-sRNA signal remain detectable after for 7 days of incubation at the tested temperatures

(Verney et al, 2020)

In brief

- **7SL-sRNA detection**: - is **sensitive** and **specific**

  - has the **advantages of indirect detection** while being **independent of the direct presence of the parasite**

  - RNA a target **stable over time**

SHORT COMMUNICATION

Transboundary and Emerging Diseases WILEY

Molecular detection of 7SL-derived small RNA is a promising alternative for trypanosomosis diagnosis

Mylène Verney[1] | Finn Grey[2] | Charlène Lemans[1] | Tristan Géraud[1] | David Berthier[3,4] | Sophie Thévenon[3,4] | Alain Rincé[5] | Aymeric Hans[1] | Liam Morrison[2] | Laurent Hébert[1]

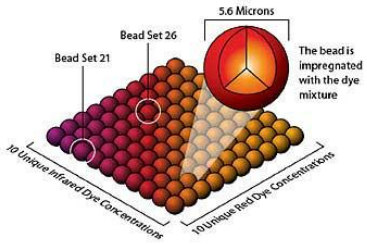
Outlooks

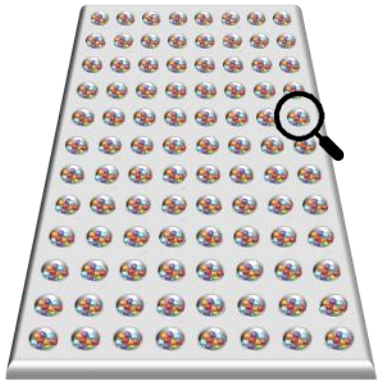
- Expand the panel of serums tested with **field samples** (chronic and acute infections)

- **Field application** (LAMP PCR or Recombinase Polymerase Amplification + LFD)?

MICROSPHERE-BASED IMMUNOASSAY (Luminex®)

1. Beads coupled with antigens





2. 2500 beads per Ag per Well

3. Incubations

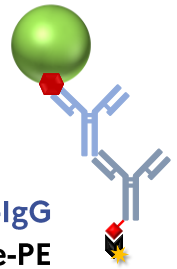
Fluorescent bead

Specific GM6 Ag

Tested serum

Biotinylated anti-IgG

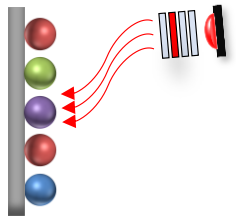
Streptavidine-PE



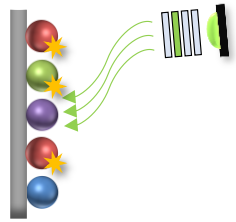
4. **Reading** Luminex® MagPix

5. Reading of fluorescence intensity

Beads recognition



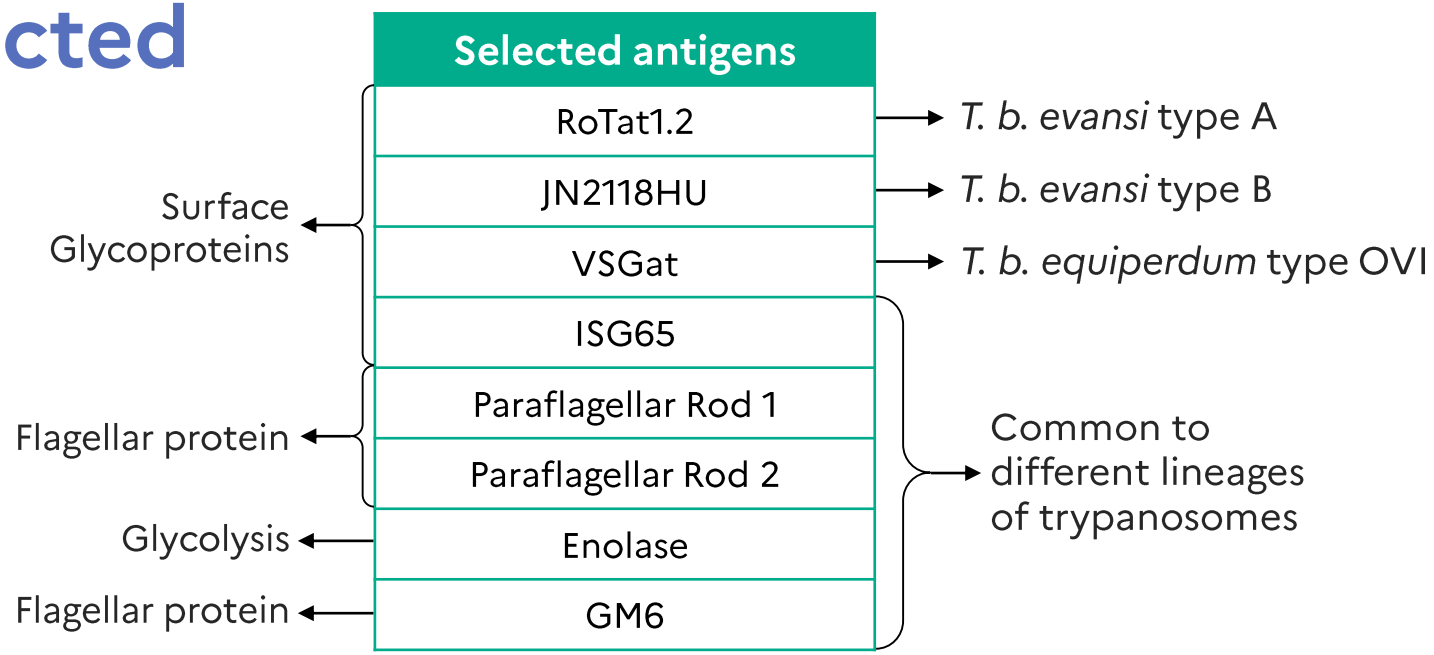
Antigens Reactivity



6. Results analysis

- Combines the advantages of flow cytometry and ELISA
- Multiplexing capability
- Requires only small sample volumes

8 antigens selected

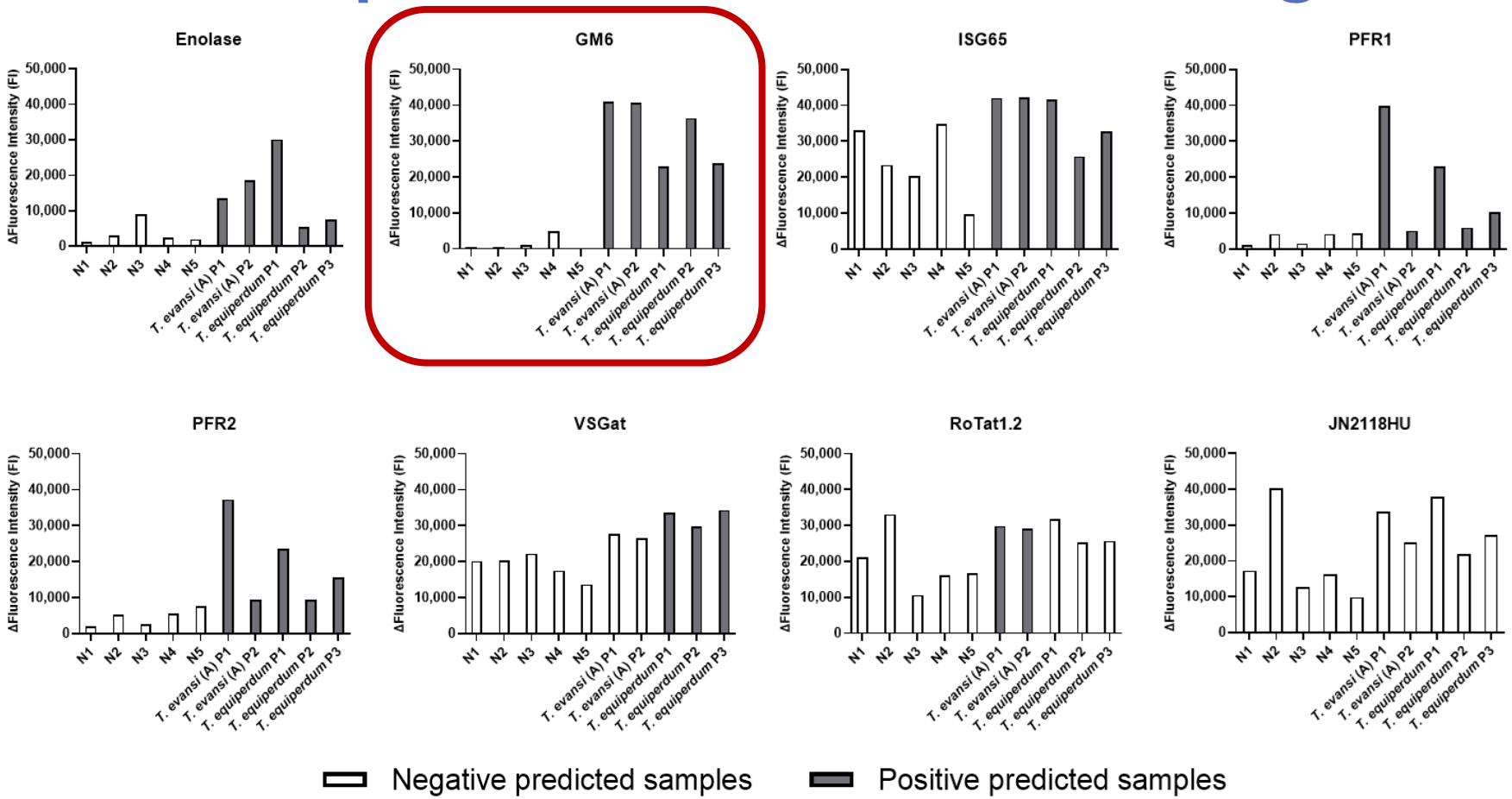


- **Heterologous production in *E. coli*** (purety > 90 %)

GeneCust
Custom Services for Research

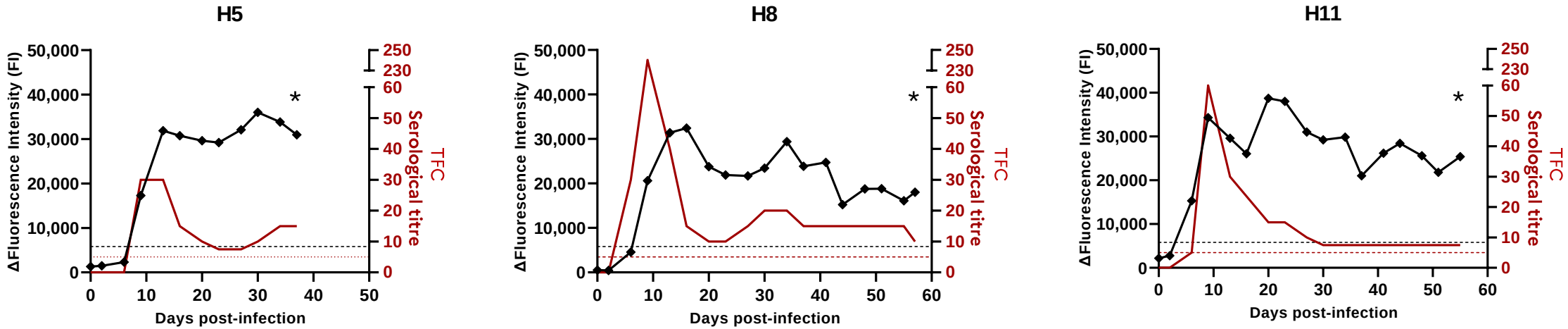
Bead coupling and performance tests

Evaluation of the performance of the 8 antigens



Selection of the GM6 antigen

Detection of anti-GM6 antibodies



- Detection **concomitant with dourine CFT**
- **Strong detectable signal** throughout the experiment

Good performance for experimentally infected horses

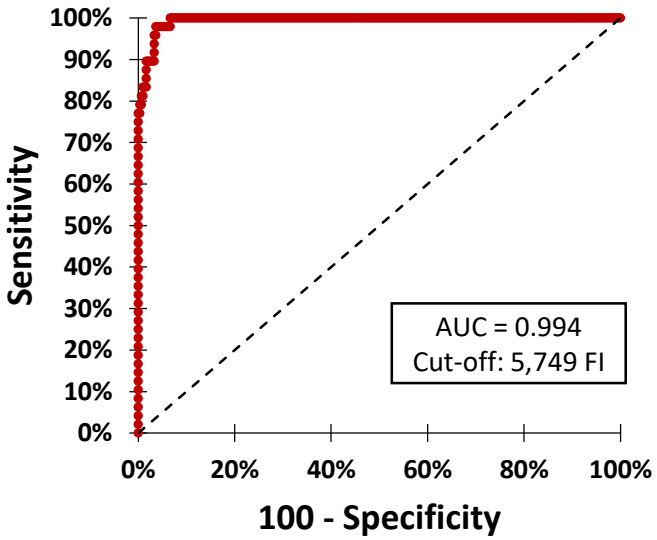
(Verney et al, 2022)

Analysis of the performance of the GM6 antigen

- 301 negative and 48 positive sera tested

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

	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



(Verney et al, 2022)

anses

In brief

scientific reports

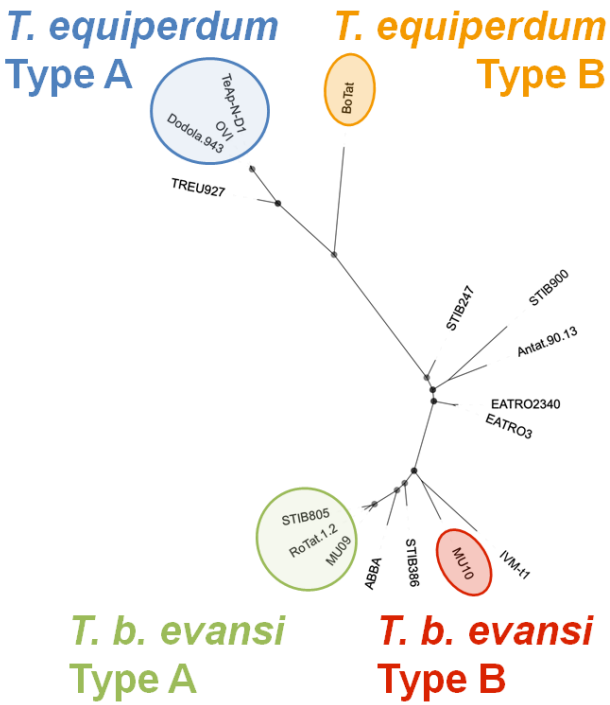
OPEN **Development of a microsphere-based immunoassay for the serological diagnosis of equine trypanosomosis**

Mylène Verney[1], Morgane Gautron[1], Charlène Lemans[1], Alain Rincé[2], Aymeric Hans[1] & Laurent Hébert[1]✉

- Identification of an antigen suitable for Luminex: **GM6**
- The **multiplexing capacity** of this technology is not yet exploited

Outlooks

- Include **clade specific antigens**
- Produce the antigens in other systems
  - e.g. *Leishmania tarentolae, Pichia pastoris…*
- Multiplex with specific antigens of **other equine diseases**



Acknowledgements

- ANSES, PhEED unit
  - Charlène Lemans
  - Morgane Gautron
  - Mylène Verney
  - Tristan Géraud
  - Aymeric Hans

EURL
European Union Reference Laboratory for
EQUINE DISEASES

- University of Caen (France)
  - Alain Rincé

UNIVERSITÉ CAEN NORMANDIE

RÉGION NORMANDIE

Centaure

- University of Edinburgh (Scotland)
  - Finn Grey
  - Liam Morrison

ROSLIN

ifce
institut français du cheval et de l'équitation

- Cirad - UMR INTERTRYP
  - David Berthier
  - Sophie Thévenon

cirad

Thank you for your attention