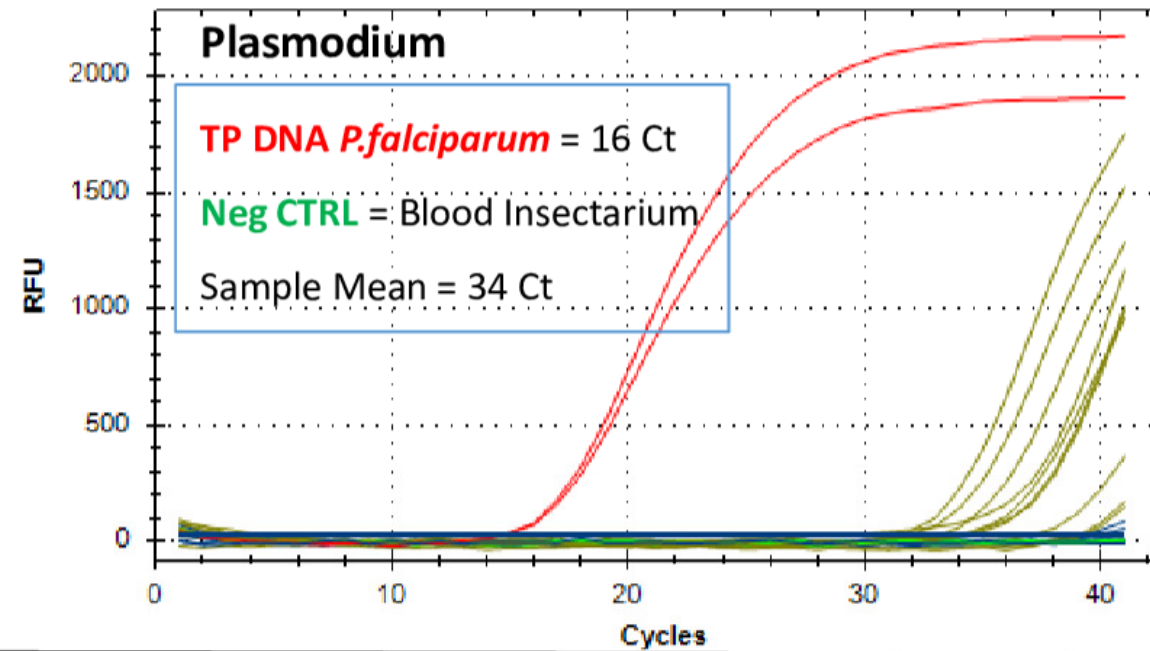
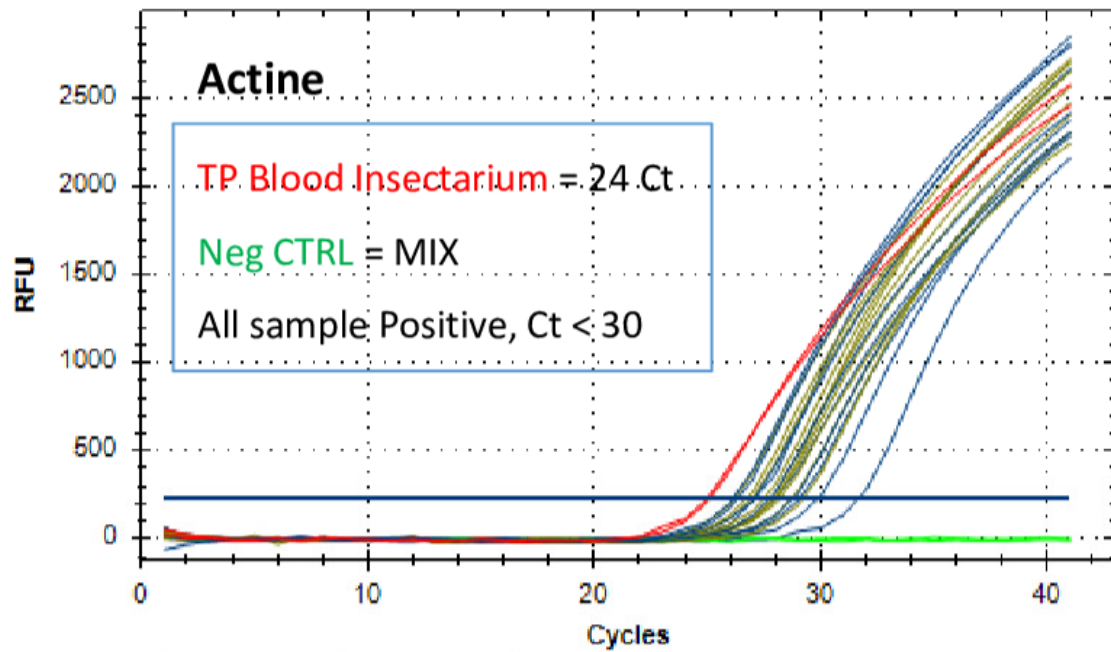


# Validation of test results

A

## qPCR results of *P. falciparum*-positive and negative RDTs DNA from the field



### Legend

- Blue curve : 10 RDTs Pf. Negative
- Green curve : 10 RDTs Pf. Positive
- Red curve : Positive control (TP)
- Light green line : Negative control (Neg CTRL)

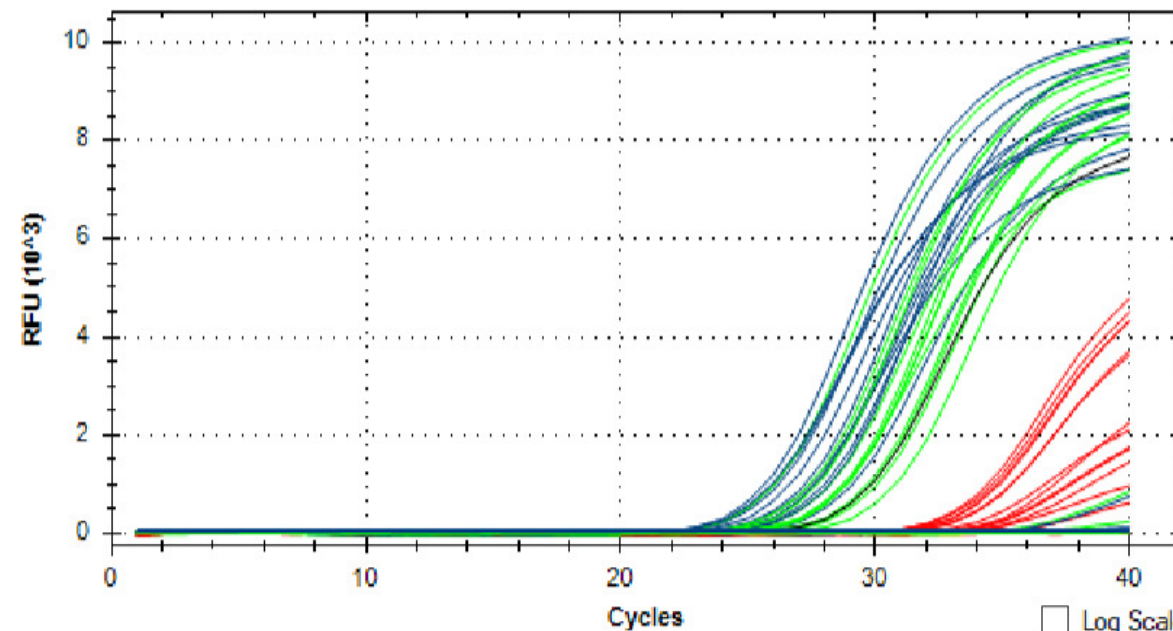
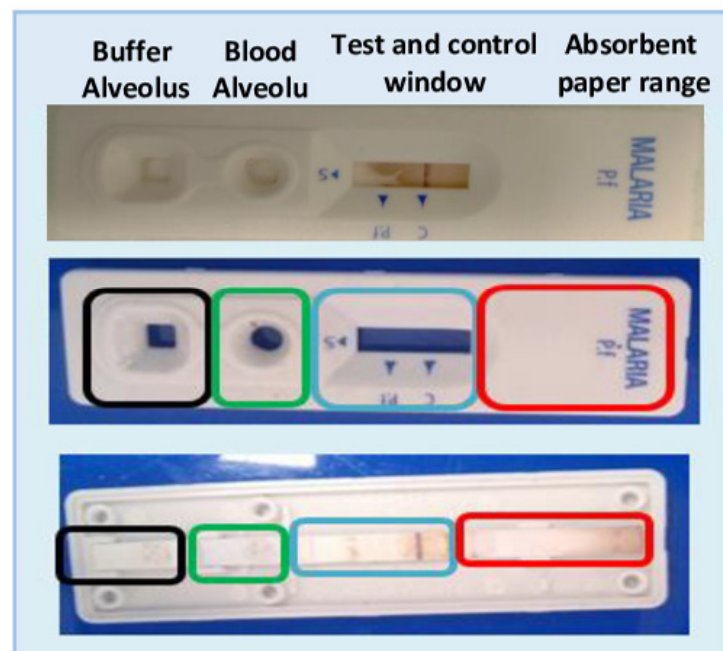
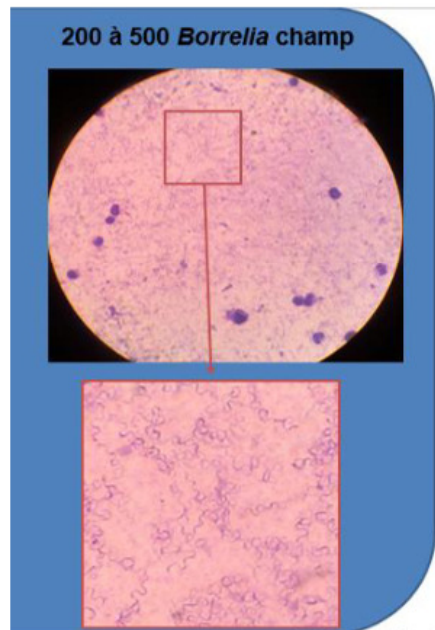
## qPCR results of blank *Plasmodium* RDTs DNA tested with *Borrelia*-positive and negative blood samples

B

### Quantification of the mixture of *Borrelia* culture strains with blood

### Dissection of the different parts of *P.falciparum* RDTs

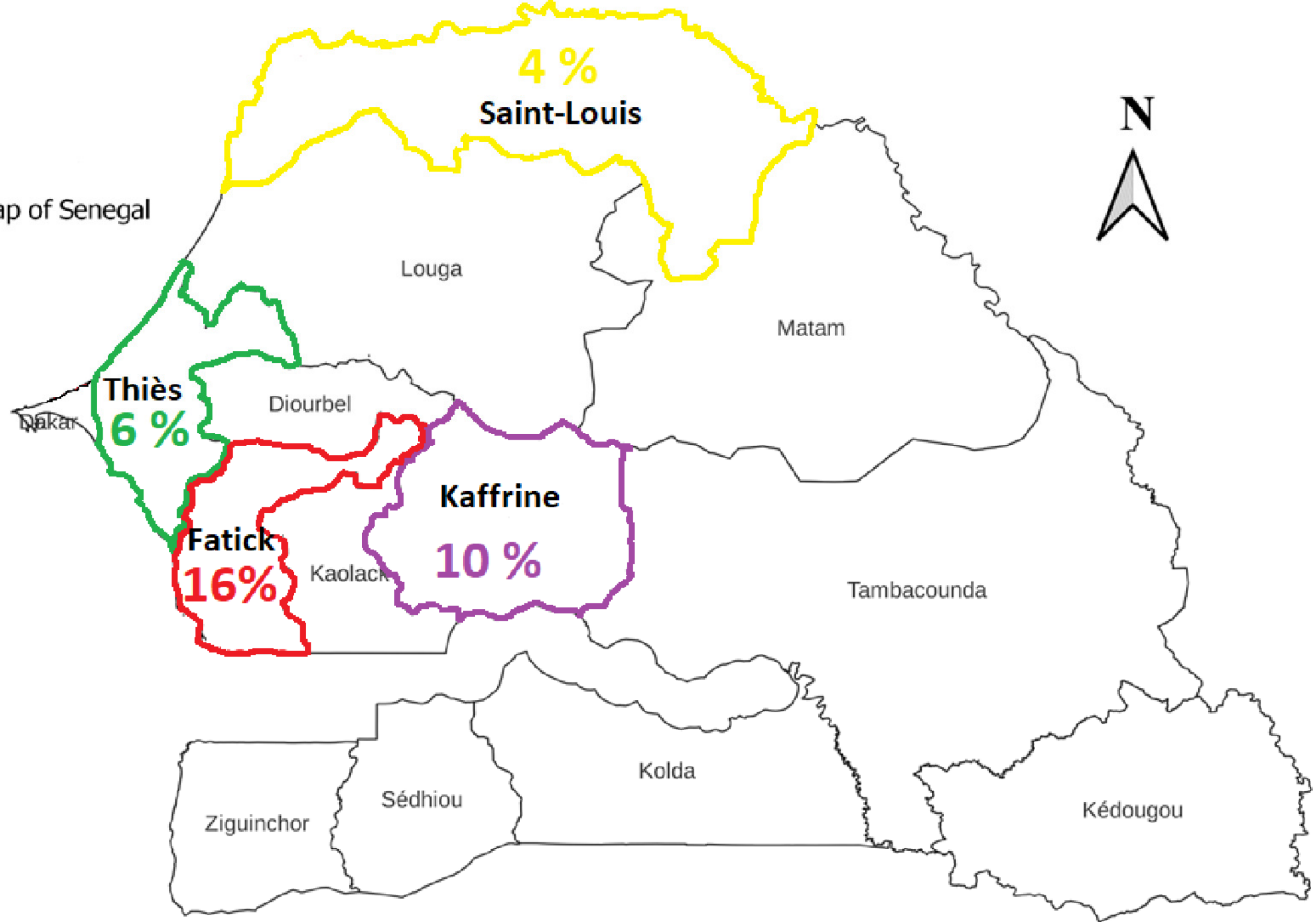
### Choice of the parts of *P.falciparum* RDTs according to bacterial load



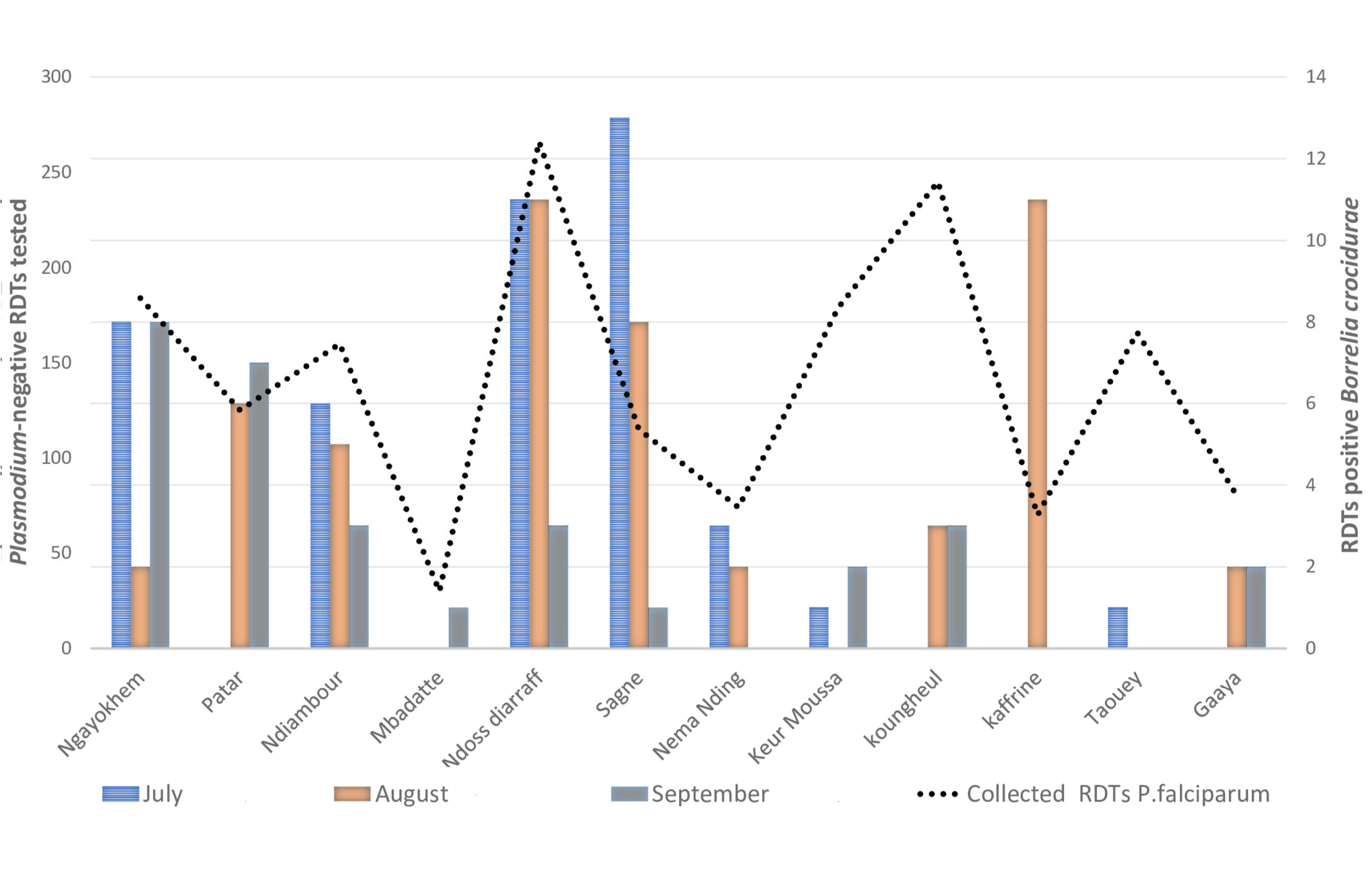
### Legend

- Blue curve : Membrane development
- Green curve : Blood deposit range
- Red curve : Absorbent paper range
- Samples : 25 RDTs Pf. positive *Borrelia*
- Control : 10 RDTs Pf. negative *Borrelia*

Map of Senegal



0 75 150 km



Supplementary Table 1: List of primers and probes of genes used for pathogen detection in this study

System name	Assay specificity	Targeted gene	Primer sequences	Reference
	<i>Borrelia</i> sp.	<i>ITS4</i>	Bor_ITS4_F (5'-GGCTTCGGGTCTACCACATCTA-3') Bor_ITS4_R (5'-CCGGGAGGGGAGTGAAATAG-3') Bor_ITS4_P (6FAM-TGCAAAAGGCACGCCATCACC)	38
	<i>B. crocidurae</i>	<i>GlpQ</i>	Bcroci_glpQ_F (5'-CCTTGGATACCCCAAATCATC-3') Bcroci_glpQ_R (5'-GGCAATGCATCAATTCTAAAC-3') Bcroci_glpQ_P (6FAM-ATGGACAAATGACAGGTCTTAC –MGB)	6
Real Time Quantitative	Bartonella sp.	<i>ITS2</i>	Barto_ITS2_F (5'-GGGGCCGTAGCTCAGCTG-3') Barto_ITS2_R (5'-TGAATATATCTTCTCTTCAATTC-3') Barto_ITS2_P (6FAM-CGATCCCCTCCGGCTCCACCA)	38
PCR	Anaplasmatatacae	<i>qANA</i>	TtAna_F (5'-TGACAGCGTACCTTTTGCAT-3') TtAna_R (5'-GTAACAGGTTCCGGTCCCA-3') TtAna_P (6FAM-GGATTAGACCCGAAACCAAG)	38
	<i>Coxiella burnetti</i>	<i>ISS11</i>	CB_IS1111_0706F (5'-CAAGAAACGTATCGCTGTGGC-3') CB_IS1111_0706R (5'-CACAGAGCCACCGTATGAATC-3') CB_IS1111_0706P (6FAM- CCGAGTTTCGAAACAATGAGGGCTG)	38
	<i>Rickettsia</i>	<i>RKND</i>	RKND03_F (5'-GTGAATGAAAGATTACACTATTTAT-3') RKND03_R (5'-GTATCTTAGCAATCATTCTAATAGC-3') RKND03_P (6FAM-CTATTATGCTTGCGGCTGTCGGTTC)	38
Standard PCR	<i>Borrelia</i> sp.	<i>FlaB</i>	B_F1 (5'-TAATACGTCAGCCATAAATGC-3') B_R1 (5'-GCTCTTTGATCAGTTATCATTC-3')	34

Supplementary Table 2: Optimisation of malaria RDTs *P. falciparum* fragments using DNA extraction load.

Sample tested	Numbers of RDT tested	Ag detection strip	<i>Actin</i>			<i>Plasmodium</i>		<i>Borrelia</i>		
			RDT frag S <b>Pos</b> (Ct mean)	RDT frag N <b>Pos</b> (Ct mean)	RDT frag F <b>Pos</b> (Ct mean)	<b>Pos</b> (Ct mean)	RDT frag S <b>Pos</b> (Ct mean)	RDT frag N <b>Pos</b> (Ct mean)	RDT frag F <b>Pos</b> (Ct mean)	
<b>1) Field SD BIOLINE malaria RDT <i>P. falciparum</i> with positive and negative band test</b>										
Positive <i>Plasmodium</i>	10	pos	10/10	(25.30)		7/10	(33.29)	**	**	**
Negative <i>Plasmodium</i>	10	neg	9/10	25.73)		0/10	(N/A)	**	**	**
<b>2) Black SD BIOLINE malaria RDTs <i>P. falciparum</i> tested with <i>Borrelia</i>-positive and negative blood</b>										
<i>Borrelia</i> negative	10	neg	9/10 (31.24)	9/10 (31.34)	2/10 (33.13)	**	0/10 (N/A)	0/10 (N/A)	0/10 (N/A)	0/10 (N/A)
<i>Borrelia</i> positive	25	neg	25/25 (30.07)	25/25 (29.61)	7/25 (35.10)	**	25/25 (25.36)	25/25 (24.01)	24/25 (32.20)	

- Antigen (Ag) detection is considered positive (pos) if both control and test *P. falciparum* bands are visible and negative (neg) if only the control band is visible (Supplementary Figure 1).

- DNA detection by qPCR on malaria RDTs *P. falciparum* is expressed as cycle threshold (Ct) values and N/A is defined as a negative result with no assigned Ct value.

- The malaria RDTs *P. falciparum* fragments (frag) used were the blood deposit portion (S), the nitrocellulose strip (N) and the final absorbent paper (F) (Supplementary Figure 1).

\*\* Malaria RDT *P. falciparum* DNA not tested.