

Supplementary Materials

New molecular approach for the detection of Kinetoplastida parasites of medical and veterinary interest

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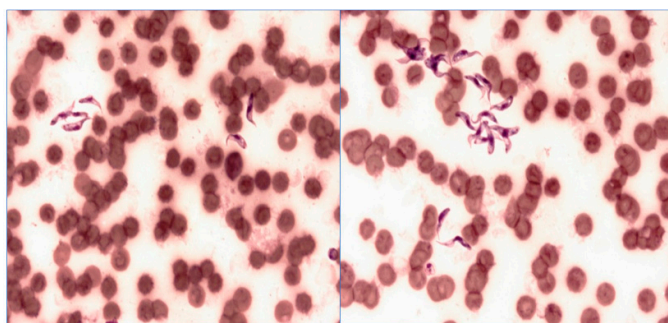


Figure S1: *Trypanosoma congolense* in a dog from Cote d'Ivoire; Microscopy G10X100; There were parasite/mL of blood

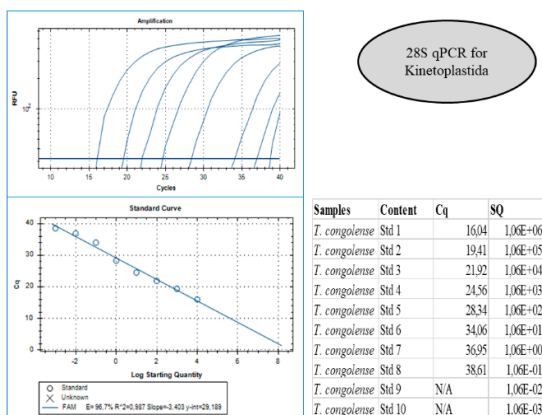


Figure S2: Standard curve and the detection limit for the TaqMan qPCR 28S LSU for Kinetoplastida

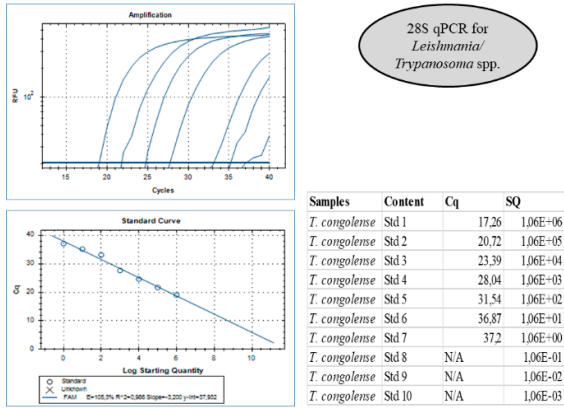


Figure S3: Standard curve and the detection limit for the TaqMan qPCR 28S LSU *Leishmania*/
Trypanosoma spp.

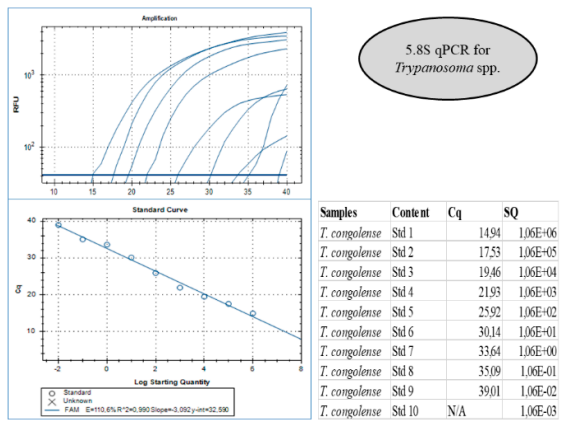


Figure S4: Standard curve and the detection limit for the TaqMan qPCR 5.8S rRNA for *Trypanosoma*
spp.

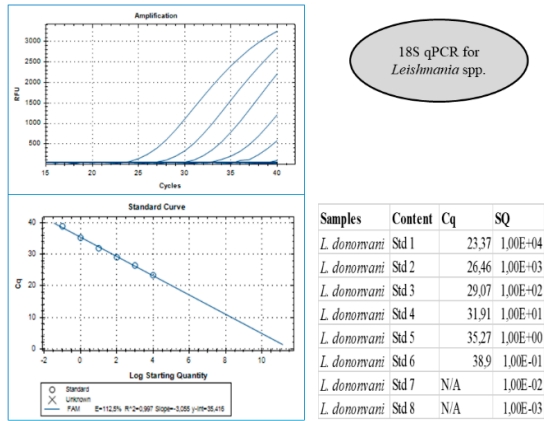


Figure S5: Standard curve and the detection limit for the TaqMan qPCR 18S SSU *Leishmania* spp.

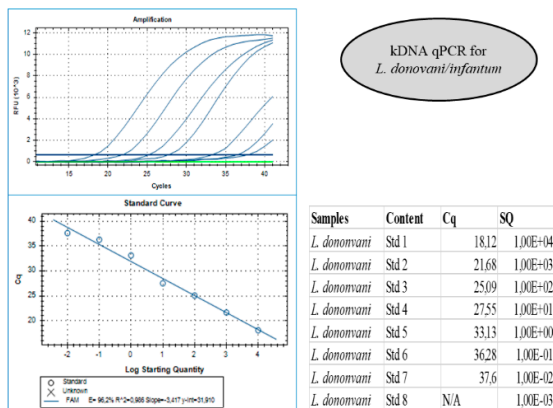


Figure S6: Standard curve and the detection limit for the TaqMan qPCR-kDNA for *Leishmania donovani* complex

Table S1. Positive (*) and negative control DNA used for sensitivity and specificity determination of designed oligonucleotides

N°	Organism	Name
1	Protozoa	<i>Leishmania infantum</i> *
2	Protozoa	<i>Leishmania major</i> *
3	Protozoa	<i>Leishmania donovani</i> *
4	Protozoa	<i>Leishmania guyanensis</i> *
5	Protozoa	<i>Trypanosoma evansi</i> *
7	Protozoa	<i>Trypanosoma brucei brucei</i> *
8	Protozoa	<i>Trypanosoma brucei gambiense</i>
9	Protozoa	<i>Trypanosoma brucei gambiense</i> biyamina groupe II*
10	Protozoa	<i>Trypanosoma congolense</i> IL 3000*
12	Protozoa	<i>Trypanosoma vivax</i> *
14	Protozoa	<i>Trypanosoma cruzi</i> CL Brunner*

15	Protozoa	<i>Trypanosoma cruzi</i> (Dog KIM)*
16	Protozoa	<i>Leptomonas</i> spp. *
17	Bacteria	<i>Rickettsia montanensis</i>
18	Lice	Head lice (<i>Pediculus humanus capitis</i>)
19	Bacteria	<i>Ehrlichia canis</i>
20	Bacteria	<i>Coxiella burnetii</i>
21	Bacteria	<i>Borrelia recurrentis</i>
22	Bacteria	<i>Staphylococcus hominis</i>
23	Bacteria	<i>Asaia bogorensis</i>
24	Piroplasm	<i>Babesia canis</i> (Moocky)
25	Bacteria	<i>Haemophilus influenzae</i>
27	Bacteria	<i>Asaia</i> C2
28	Bacteria	<i>Wolbachia</i> sp.
29	Bacteria	<i>Anaplasma phagocytophilum</i>
30	Bacteria	<i>Enterobacter aerogenes</i>
31	Bacteria	<i>Streptococcus pneumoniae</i>
32	Bacteria	<i>Salmonella enterica</i>
33	Bacteria	<i>Citrobacter koseri</i>
34	Bacteria	<i>Gardnerella vaginalis</i>
35	Bacteria	<i>Streptococcus pyogenes</i>
36	Protozoa	<i>Plasmodium</i> sp.
37	Bacteria	<i>Rickettsia typhi</i>
38	Bacteria	<i>Enterococcus faecium</i>
39	Bacteria	<i>Streptococcus agalactiae</i>
40	Bacteria	<i>Rickettsia conorii</i>
41	Tick	<i>Rhipicephalus microplus</i>
42	Bovine	BA286 cell line
43	Dog	DH62 cell line
44	Human	HL60 cell line (<i>Homo sapiens</i>)
45	Donkey	ANE 4 (<i>Equus asinus</i>)
46	Horse	CV.G 22 (<i>Equus caballus</i>)
47	Flea	<i>Ctenocephalides felis</i>
48	Bedbugs	<i>Cimex lectularius</i>
49	Tick	<i>Hyalomma marginatum</i>
50	Tick	<i>Amblyomma variegatum</i>
51	Dog	DH62 dog cell line (<i>Canis lupus familiaris</i>)
52	Protozoa	<i>Hepatozoon canis</i>
53	Protozoa	<i>Leptomonas saimouri</i>
54	Piroplasm	<i>Theileria equi</i>
55	Bacteria	<i>Rickettsia massiliae</i>
56	Bacteria	<i>Staphylococcus haemolyticus</i>
57	Bacteria	<i>Staphylococcus aureus</i>
58	Bacteria	<i>Rickettsia felis</i>
59	Bacteria	<i>Stenotrophomonas maltophilia</i>
60	Bacteria	<i>Acinetobacter</i> sp.
61	Bacteria	<i>Enterobacter aerogenes</i>

Table S2. Ct values repeatability and reproducibility of the real time qPCR assays.

<i>L. donovani</i> load	kDNA <i>Leishmania donovani</i> complex qPCR						Intra assay variations of Ct values					Inter assay variations of Ct values				
	Replicate 1	Replicate 2	Mean	SD	CV %	Assay	Assay		Mean	SD	CV %	Assay	Assay	Mean	SD	CV %
							1	2								
1,00E+04	18,12	17,88	18	0,17	0,94	18	18,66	18,33	0,47	2,55	18	18,66	18,33	0,47	2,55	
1,00E+03	21,68	21,36	21,52	0,23	1,05	21,52	21,22	21,37	0,21	0,99	21,52	21,22	21,37	0,21	0,99	
1,00E+02	25,09	24,9	24,995	0,13	0,54	24,995	23,99	24,49	0,71	2,90	24,995	23,99	24,49	0,71	2,90	
1,00E+01	27,55	27,71	27,63	0,11	0,41	27,63	26,93	27,28	0,49	1,81	27,63	26,93	27,28	0,49	1,81	
1,00E+00	33,13	33,01	33,07	0,08	0,26	33,07	33,41	33,24	0,24	0,72	33,07	33,41	33,24	0,24	0,72	
1,00E-01	36,28	37,04	36,66	0,54	1,47	36,66	36,47	36,57	0,13	0,37	36,66	36,47	36,57	0,13	0,37	

1,00E-02	37,6	37,99	37,795	0,28	0,73	37,795	38,11	37,95	0,22	0,59
1,00E-03	-	-	-	-	-	-	-	-	-	-

18S *Leishmania* spp. qPCR

<i>L. donovani</i> load	Intra assay variations of Ct values						Inter assay variations of Ct values				
	Replicate 1	Replicate 2	Mean	SD	CV %	Assay		Mean	SD	CV %	
						1	2				
1,00E+04	23,37	22,98	23,175	0,28	1,19	23,175	22,84	23,01	0,24	1,03	
1,00E+03	26,46	25,89	26,175	0,40	1,54	26,175	26,33	26,25	0,11	0,42	
1,00E+02	29,07	28,94	29,005	0,09	0,32	29,005	29,09	29,05	0,06	0,21	
1,00E+01	31,91	32,01	31,96	0,07	0,22	31,96	31,07	31,52	0,63	2,00	
1,00E+00	35,27	36	35,635	0,52	1,45	35,635	38,65	37,14	2,13	5,74	
1,00E-01	38,9	39,44	39,17	0,38	0,97	39,17	39,82	39,50	0,46	1,16	
1,00E-02	-	-	-	-	-	-	-	-	-	-	
1,00E-03	-	-	-	-	-	-	-	-	-	-	

5.8S *Trypanosoma* spp. qPCR

<i>T. congolense</i> load	Intra assay variations of Ct values						Inter assay variations of Ct values				
	Replicate 1	Replicate 2	Mean	SD	CV %	Assay		Mean	SD	CV %	
						1	2				
1,06E+06	14,94	15,34	15,14	0,28	1,87	15,14	16,12	15,63	0,69	4,43	
1,06E+05	17,53	18,89	18,21	0,96	5,28	18,21	20,31	19,26	1,48	7,71	
1,06E+04	19,46	20,43	19,945	0,69	3,44	19,945	22,21	21,08	1,60	7,60	
1,06E+03	21,93	22,76	22,345	0,59	2,63	22,345	25,31	23,83	2,10	8,80	
1,06E+02	25,92	26,33	26,125	0,29	1,11	26,125	27,96	27,04	1,30	4,80	
1,06E+01	30,14	31	30,57	0,61	1,99	30,57	29,88	30,23	0,49	1,61	
1,06E+00	33,64	3,36E+01	33,6	0,06	0,17	33,6	32,64	33,12	0,68	2,05	
1,06E-01	35,09	36,21	35,65	0,79	2,22	35,65	34,11	34,88	1,09	3,12	
1,06E-02	39,01	39,14	39,075	0,09	0,24	39,075	38,39	38,73	0,48	1,25	
1,06E-03	-	-	-	-	-	-	-	-	-	-	

28S *Leishmania/Trypanosoma* spp. qPCR

<i>T. congolense</i> load	Intra assay variations of Ct values						Inter assay variations of Ct values				
	Replicate 1	Replicate 2	Mean	SD	CV %	Assay		Mean	SD	CV %	
						1	2				
1,06E+06	17,26	18,56	17,91	0,92	5,13	17,91	18,19	18,05	0,20	1,10	
1,06E+05	20,72	22	21,36	0,91	4,24	21,36	22,3	21,83	0,66	3,04	
1,06E+04	23,39	23,96	23,675	0,40	1,70	23,675	23,55	23,61	0,09	0,37	
1,06E+03	28,04	29,14	28,59	0,78	2,72	28,59	30	29,30	1,00	3,40	
1,06E+02	31,54	32,66	32,1	0,79	2,47	32,1	33,33	32,72	0,87	2,66	
1,06E+01	36,87	37,8	37,335	0,66	1,76	37,335	38,09	37,71	0,53	1,42	
1,06E+00	37,2	38,33	37,765	0,80	2,12	37,765	39,34	38,55	1,11	2,89	
1,06E-01	-	-	-	-	-	-	-	-	-	-	
1,06E-02	-	-	-	-	-	-	-	-	-	-	
1,06E-03	-	-	-	-	-	-	-	-	-	-	

28S Kinetoplastida spp. qPCR

<i>T. congolense</i> load	Intra assay variations of Ct values						Inter assay variations of Ct values				
	Replicate 1	Replicate 2	Mean	SD	CV %	Assay		Mean	SD	CV %	
						1	2				
1,06E+06	16,04	15,68	15,86	0,25	1,61	15,86	16,51	16,19	0,46	2,84	
1,06E+05	19,41	18,9	19,155	0,36	1,88	19,155	18,58	18,87	0,41	2,15	

1,06E+04	21,92	21,04	21,48	0,62	2,90	21,48	22,04	21,76	0,40	1,82
1,06E+03	24,56	23,95	24,255	0,43	1,78	24,255	27,65	25,95	2,40	9,25
1,06E+02	28,34	28	28,17	0,24	0,85	28,17	27,9	28,04	0,19	0,68
1,06E+01	34,06	32,47	33,265	1,12	3,38	33,265	30,14	31,70	2,21	6,97
1,06E+00	36,95	35,11	36,03	1,30	3,61	36,03	31,69	33,86	3,07	9,06
1,06E-01	38,61	37,78	38,195	0,59	1,54	38,195	33,21	35,70	3,52	9,87
1,06E-02		39,5	39,5	-	-	39,5	38,43	38,97	0,76	1,94
1,06E-03	-	-	-	-	-	-	-	-	-	-

Table S3. Individual results for samples screened by the qPCR tools and typing.

Sample's ID	Species	Country	28 S Kineto	28 S Leish- Tryp	5.8S Tryp	18S Leish	kDNA L. dono Cplx	kDNA L. inf	Typing
1	Dog	Algeria	+	+	-	+	+	+	<i>L. infantum</i>
2	Dog	Algeria	+	+	-	-	-	-	-
3	Dog	Algeria	+	-	-	-	-	-	-
3	Dog	Algeria	+	-	-	-	-	-	<i>B. caudatus</i>
4	Dog	Algeria	+	-	-	-	-	-	<i>B. caudatus</i>
5	Dog	Algeria	+	-	-	-	-	-	<i>B. caudatus</i>
6	Dog	Algeria	+	-	-	-	-	-	-
7	Dog	Algeria	-	+	-	-	-	-	-
8	Dog	Algeria	+	-	-	-	-	-	-
9	Dog	Algeria	+	-	-	-	-	-	-
10	Dog	Algeria	+	+	-	-	-	-	-
11	Dog	Algeria	-	+	-	+	+	+	<i>L. infantum</i>
12	Dog	Algeria	+	+	-	-	+	+	<i>L. infantum</i>
13	Dog	Algeria	+	-	-	-	+	+	<i>L. infantum</i>
14	Dog	Algeria	+	+	+	-	-	-	<i>T. evansi</i>
15	Dog	Algeria	-	+	-	-	-	-	-
16	Dog	Algeria	+	+	+	-	-	-	<i>T. evansi</i>
17	Dog	Algeria	+	+	+	-	-	-	<i>T. evansi</i>
S1	Dog	Algeria	+	-	-	-	-	-	-
S11	Dog	Algeria	+	-	-	+	+	+	<i>L. infantum</i>
S14	Dog	Algeria	+	+	+	-	-	-	<i>T. congolense</i>
S17	Dog	Algeria	+	+	+	-	-	-	<i>T. congolense</i>
S26	Dog	Algeria	+	+	-	+	+	-	<i>L. donovani/L. infantum</i>
S27	Dog	Algeria	+	+	-	+	+	+	<i>L. donovani/L. infantum</i>
S33	Dog	Algeria	+	+	-	+	+	+	<i>L. infantum</i>
S41	Dog	Algeria	+	+	+	-	-	-	<i>T. evansi</i>
S49	Dog	Algeria	+	+	+	-	-	-	<i>T. evansi</i>
S50	Dog	Algeria	+	+	-	+	+	+	<i>L. infantum</i>
S54	Dog	Algeria	+	-	-	-	+	+	<i>L. infantum</i>
S73	Dog	Algeria	+	+	-	-	-	-	No sequence
B5	Dog	Algeria	+	+	+	-	-	-	<i>T. evansi</i>
B6	Dog	Algeria	+	+	+	-	-	-	<i>T. evansi</i>
T3	Dog	Algeria	+	+	-	+	+	+	<i>L. donovani/L. infantum</i>
ANE	Equid	Algeria	+	+	-	+	+	+	<i>L. infantum</i>
CHEVAL	Equid	Algeria	+	+	-	+	+	+	<i>L. infantum</i>
CMT 21	Dog	French Guiana	+	-	-	+	+	+	<i>L. infantum</i>
CMT 72	Dog	French Guiana	+	-	-	-	-	-	-
CMT 78	Dog	French Guiana	-	+	+	-	-	-	No sequence
CMT 80	Dog	French Guiana	+	+	-	+	+	+	<i>L. infantum</i>
CMT 86	Dog	French Guiana	-	+	+	-	-	-	No sequence
CMT 89	Dog	French Guiana	-	-	+	-	-	-	No sequence
CMT 94	Dog	French Guiana	+	-	-	-	-	-	No sequence
CMT 95	Dog	French Guiana	+	+	+	+	+	+	<i>L. infantum</i>
B2	Monkey	French Guiana	+	+	-	+	+	+	<i>L. infantum</i>
B6	Monkey	French Guiana	+	+	-	+	+	+	<i>L. infantum</i>

B8	Monkey	French Guiana	+	+	-	+	-	-	<i>L. guyanensis</i>
B9	Monkey	French Guiana	-	-	-	+	-	-	-
EA16	Dog	Cote d'Ivoire	-	-	+	-	-	-	<i>T. congolense</i>
EA33	Dog	Cote d'Ivoire	+	+	+	-	+	+	<i>T. congolense/ L. infantum</i>
EA40	Dog	Cote d'Ivoire	+	+	+	+	+	-	-
EA56	Dog	Cote d'Ivoire	-	-	+	-	-	-	<i>T. congolense</i>
EA64	Dog	Cote d'Ivoire	+	+	+	+	+	+	<i>T. congolense/ L. infantum</i>
EA79	Dog	Cote d'Ivoire	+	+	-	+	+	+	<i>L. infantum</i>
EA95	Dog	Cote d'Ivoire	+	+	-	+	+	+	<i>L. infantum</i>

No sequence: means PCR + but not sequence obtained