

Supplementary Table 1: Data of PCR methods used in this study for DNA quality control (A), pathogen screening (B-E) and confirming *Borrelia* genospecies identity (F-I).

PCR code	Target group	Target gene	Primer (probe) name	Primer sequence (5'-3')	Amplicon length (bp)	Thermocycling profile	Reference
A	Ixodidae	16 rRNA	16S+1 16S-1	CTG CTC AAT GAT TTT TTA AAT TGC TGT GG CCG GTC TGA ACT CAG ATC AAG T	~460	95 °C for 5 min; 40× (95 °C for 40 s; 51 °C for 1 min; 72 °C for 1 min); 72 °C for 10 min	Black and Piesman, 1994
B	<i>Borrelia burgdorferi</i> s.l.	5S-23S ITS	B5S-borseq B23S-borseq	GAG TTC GCG GGA GAG TAG GTT ATT GCC TCA GGG TAC TTA GAT GGT TCA CTT CC	~450	94 °C for 5 min; 10× 'touchdown' (94 °C for 20 s, 70 °C for 30 s (dropping 1 °C per cycle), 72 °C for 30 s); 40× (94 °C for 20 s, 60 °C for 30 s, 72 °C for 30s); 72 °C for 7 min	Heylen et al., 2013
C	Piroplasms	18S rDNS	BJ1 BN2	GTC TTG TAA TTG GAA TGA TGG TAG TTT ATG GTT AGG ACT ACG	~500	95 °C for 10 min; 40× (95 °C for 30 s; 54 °C for 30 s; 72 °C for 40 s); 72 °C for 5 min	Casati et al., 2006
D	<i>Rickettsia</i> spp.	gltA	RpCs.877p RpCs.1258n	GGG GGC CTG CTC ACG GCG G ATT GCA AAA AGT ACA GTG AAC A	~380	95 °C for 5 min; 40× (95 °C for 20 s; 48 °C for 30 s; 72 °C for 1 min); 72 °C for 5 min	Regnery et al., 1991
E	<i>Anaplasma phagocytophilum</i>	msp2	ApMSP2f ApMSP2r (ApMSP2p)	ATG GAA GGT AGT GTT GGT TAT GGT ATT TTG GTC TTG AAG CGC TCG TA FAM-TGG TGC CAG GGT TGA GCT TGA GAT TG-TAMRA	~77	95 °C for 20 s; 40× (95 °C for 3 s; 60 °C for 30 s)	Courtney et al., 2004
F	<i>Borrelia burgdorferi</i> s.l.	OspA outer	V1a V1b R1 R37	GGG AAT AGG TCT AAT ATT AGC GGG GAT AGG TCT AAT ATT AGC CAT AAA TTC TCC TTA TTT TAA AGC CCT TAT TTT AAA GCG GC	~840	95 °C for 5 min; 40× (94 °C for 40 s; 48 °C for 1 min; 72 °C for 1 min); 72 °C for 10 min	Michel et al., 2003
		OspA nested	V3a V3b R1 R37	GCC TTA ATA GCA TGT AAG C GCC TTA ATA GCA TGC AAG C CAT AAA TTC TCC TTA TTT TAA AGC CCT TAT TTT AAA GCG GC	~800	95 °C for 5 min; 40× (94 °C for 40 s; 48 °C for 1 min; 72 °C for 1 min); 72 °C for 10 min	
G		OspA outer	OspA-N1 OspA-C1	GAG CTT AAA GGA ACT TCT GAT AA GTA TTG TTG TAC TGT AAT TGT	~560	95 °C for 5 min; 40× (95 °C for 30 s; 45 °C for 30 s; 72 °C for 1 min); 72 °C for 5 min	

	<i>Borrelia burgdorferi</i> s.l.	OspA nested	OspA-N2 OspA-C2	ATG GAT CTG GAG TAC TTG AA CTT AAA GTA ACA GTT CCT TCT	~351	95 °C for 5 min; 40× (95 °C for 30 s; 45 °C for 30 s; 72 °C for 1 min); 72 °C for 5 min	Guy, Stanek 1991
H	<i>Borrelia burgdorferi</i> s.l.	OspA outer	external(+) external(-)	AAA AAA TAT TTA TTG GGA ATA GG GT TTT TTT GCT GTT TAC ACT AAT TGT TAA	~702	95 °C for 5 min; 40× (95 °C for 30 s; 45 °C for 30 s; 72 °C for 1 min); 72 °C for 5 min	Guttman et al., 1996
		OspA nested	internal(+) internal(-)	GGA GTA CTT GAA GGC G GCT TAA AGT AAC AGT TCC	~351	95 °C for 5 min; 10× (95 °C for 30 s; 60 °C for 30 s; 72 °C for 1 min); 10× (95 °C for 30 s; 55 °C for 30 s; 72 °C for 1 min); 10× (95 °C for 30 s; 50 °C for 30 s; 72 °C for 1 min); 5× (95 °C for 30 s; 45 °C for 30 s; 72 °C for 1 min); 72 °C for 5 min	
I	<i>Borrelia burgdorferi</i> s.l.	OspC outer	external(+) external(-)	AAA GAA TAC ATT AAG TGC GAT ATT GGG CTT GTA AGC TCT TTA ACT G	~595	95 °C for 5 min; 40× (95 °C for 30 s; 53 °C for 40 s; 72 °C for 1 min); 72 °C for 10 min	Wang et al., 1999
		OspC semi-nested1	external(+) internal(-)	AAA GAA TAC ATT AAG TGC GAT ATT CAA TCC ACT TAA TTT TTGTGT TAT TAG	~340	95 °C for 5 min; 40× (95 °C for 30 s; 53 °C for 40 s; 72 °C for 1 min); 72 °C for 10 min	
		OspA semi-nested2	internal(+) external(-)	TTG TTA GCA GGA GCT TAT GCA ATA TC GGG CTT GTA AGC TCT TTA ACT G	~314	95 °C for 5 min; 40× (95 °C for 30 s; 53 °C for 40 s; 72 °C for 1 min); 72 °C for 10 min	

References

- Black WC, Piesman J. Phylogeny of hard and soft-tick taxa (Acari: Ixodida) based on mitochondrial 16S rDNA sequences. *Proc. Nat. Acad. Sci. USA* 1994;91:10034–10038.
- Casati S, Sager H, Gern L, Piffaretti JC. Presence of potentially pathogenic *Babesia* sp. for human in *Ixodes ricinus* in Switzerland. *Ann Agric Environ Med*. 2006;13:65-70.
- Courtney JW, Kostelnik LM, Zeidner NS, Massung RF. Multiplex real-time PCR for detection of *Anaplasma phagocytophilum* and *Borrelia burgdorferi*. *J Clin Microbiol*. 2004;42:3164-8. doi: 10.1128/JCM.42.7.3164-3168.2004.
- Guttman DS, Wang PW, Wang IN, Bosler EM, Luft BJ, Dykhuizen DE. Multiple infections of *Ixodes scapularis* ticks by *Borrelia burgdorferi* as revealed by single-strand conformation polymorphism analysis. *J Clin Microbiol*. 1996;34:652-6. doi: 10.1128/jcm.34.3.652-656.1996.
- Guy EC, Stanek G. Detection of *Borrelia burgdorferi* in patients with Lyme disease by the polymerase chain reaction. *J Clin Pathol*. 1991 Jul;44(7):610-1. doi: 10.1136/jcp.44.7.610.
- Heylen D, Tijssse E, Fonville M, Matthysen E, Sprong H. Transmission dynamics of *Borrelia burgdorferi* s.l. in a bird tick community. *Environ Microbiol*. 2013;15:663-73. doi: 10.1111/1462-2920.12059.
- Michel H, Wilske B, Hettche G, Göttner G, Heimerl C, Reischl U, Schulte-Spechtel U, Fingerle V. An ospA-polymerase chain reaction/restriction fragment length polymorphism-based method for sensitive detection and reliable differentiation of all European *Borrelia burgdorferi* sensu lato species and OspA types. *Med Microbiol Immunol*. 2004;193:219-26. doi: 10.1007/s00430-003-0196-8.
- Regnery RL, Spruill CL, Plikaytis BD. Genotypic identification of rickettsiae and estimation of intraspecies sequence divergence for portions of two rickettsial genes. *J Bacteriol*. 1991;173:1576-89. doi: 10.1128/jb.173.5.1576-1589.1991.
- Wang IN, Dykhuizen DE, Qiu W, Dunn JJ, Bosler EM, Luft BJ. Genetic diversity of ospC in a local population of *Borrelia burgdorferi* sensu stricto. *Genetics*. 1999;151:15-30. doi: 10.1093/genetics/151.1.15.