

Table S1. *In silico* nucleotide base difference analysis of 23S rRNA sequences from various treponeme species compared to *T. pallidum* ssp. *pallidum* in the 23S rRNA target region of the *Treponema pallidum* TMA assay.

Species (Strain; NCBI Genbank Accession Number)	% base difference from <i>T. pallidum</i> ssp. <i>pallidum</i> in TMA assay target region	TMA % cross reactivity (n=3)^a
<i>T. pallidum</i> ssp. <i>endemicum</i> (Samoa F; CP007548.1)	0	100
<i>T. pallidum</i> ssp. <i>pertenue</i> (CDC2; CP002375.1)	0	100
<i>T. paraluisuniculi</i> (Cuniculi A; JX120547.1)	0	100
<i>T. phagedenis</i> (4A; AQCFO1)	18	0
<i>T. denticola</i> (OTK; AGDYO1)	27	0
<i>T. succinifaciens</i> (DSM 2489; CP002631.1)	27	NT
<i>T. primitia</i> (ZAS-2; NR_076709.1)	28	NT
<i>T. pedis</i> (T M1; AOTMO1)	28	NT
<i>T. caldarium</i> (H1; NR_076899.1)	30	NT
<i>T. azotonutricium</i> (ZAS-9; NR_076708.1)	30	NT
<i>T. socranskii</i> (ssp. <i>paredis</i> ; NZ_KE332516.1)	34	NT
<i>T. putidum</i> (OMZ758; CP009228.1)	38	NT
<i>T. brennaborensis</i> (DD5/3; NR_076878.1)	40	NT
<i>T. lecithinolyticum</i> (ATCC700322; NZ_KI260563.1)	41	NT
<i>T. maltophilum</i> (ATCC 51939; ATFF01000006.1)	59	NT

^a TMA results from Table 2. NT, not tested.