SUPPLEMENTARY MATERIAL AND METHODS

Laboratory methods

Specimens were collected in universal transport medium (UTM, Copan, Brescia, Italy) tubes and DNA was extracted using BT Chelex 100 Resin (Bio-Rad Laboratories Inc., CA, USA). The samples were tested for the presence of *T. pallidum* using two real-time diagnostic PCR assays with internal amplification controls, amplifying segments of the flaA (S1) and polA (S2) genes of *T. pallidum* and the bacterial loads were determined from these PCR assays using 10-fold dilutions of purified *T. pallidum* DNA. The bacterial load was used for comparison between typeable and non-typeable specimens. Subsequently, the specimens were stored at −20°C until they were subjected to strain typing. PCR was used to determine the number of 60-bp repeats within the arp gene as previously described (6), but with primer modifications as described by Katz et al. (14).

In brief, 5 µl of DNA was amplified in a 100 µl reaction, using 2 U Taq DNA polymerase (Platinum Taq, Invitrogen, Paisley, UK), 125 µM each dATP, dGTP, and dCTP; 250 µM dUTP, 200 nM primers, 1.5 mM MgCl2 and 1X PCR PCR reaction buffer (Invitrogen). We used the primers ARP N1 (forward) and ARP N2 (reverse) (14). Cycling conditions, including a touchdown step, consisted of an initial cycle of 94°C for 2 min, followed by 10 cycles of 94°C for 30 s; 74°C for 45 s decreasing 1°C per cycle, and 72°C for 90 s. The next step consisted of 35 cycles of 94°C for 30 s; 64°C for 45 s, and 72°C for 90 s. Final extension was achieved at 72°C for 10 min. The amplification yielded different amplicon sizes depending on the different number of 60-bp repeats within the arp gene. The number of 60-bp repeats was estimated using plasmids with a known number of repeats as references, kindly provided by Bess Charmie Godornes from the University of Washington, Seattle, USA. Further, specimens were compared to the Nichols strain of *T. pallidum* with the known amplicon size of 1155 bp, corresponding to 14 repeats.

A nested PCR was used to analyze tpr. In brief, 10 µl of DNA was amplified in a 100 µl reaction, using 2 U Taq DNA polymerase (Platinum Taq, Invitrogen), 125 µM each dATP, dGTP, and dCTP; 400 nM primers, 1.5 mM MgCl2 and 1X PCR reaction buffer (Invitrogen). We used the primers 5’ CAGGTTTTGCCGTTAAGC3’ (forward) and 5’ TCTCTATGAGACTGGCTGAAA3’ (reverse). An initial cycle of 94°C for 2 min was followed by a touchdown step, consisting of 10 cycles of 94°C for 15 s; 65°C for 15 s decreasing 1°C per cycle, and 72°C for 30 s. The next step consisted of 35 cycles of 94°C for 15 s; 55°C for 15 s, and 72°C for 30 s. Final extension was achieved at 72°C for 10 min. The PCR products were sequenced using standard methods.

REFERENCES
