Inability of One-Step Real-Time PCR To Detect
Borrelia burgdorferi DNA in Urine

We recently described successful molecular diagnosis of B. burgdorferi DNA by nested PCR in urine in patient-derived samples only after extraction with DNAzol (1).

The aim of this study was to examine whether Borrelia burgdorferi DNA can also be detected in urine by quantitative one-step real-time PCR (Q-PCR). Q-PCR was performed after extraction with DNAzol (Molecular Research Center Inc., Cincinnati, Ohio), using the same protocol as recently published, and also after extraction with the QIAamp DNA Mini kit (Qiagen, Hilden, Germany) and the High Pure PCR template preparation kit (Roche Diagnostics, Mannheim, Germany). Q-PCR was performed according to the assay method of Schwaiger et al. (2) and also by using newly designed primers and probes derived from the flagellin gene (B. burgdorferi sensu stricto: TaqMan probe, 5'-FAM-ATT GCT GAT CAA GCT CAA TAT AAC CAA ATG CAC A-TAMRA 3'; forward primer, 5'-TGA AAT AGA GCA ACT TAC AGA CGA AAT T-3'; reverse primer, 5'-CAT TTT GAG AAG CAG ATT TGT TTG A-3'). For the latter PCR, 5 μl of DNAzol-extracted DNA or 10 μl of QIAamp- or Roche-extracted DNA, respectively, was mixed with 12.6 μl of 2× TaqMan buffer, 200 nM TaqMan probe, 600 nM (each) primer, and H2O to a final volume of 25 μl. Amplification was performed on a GeneAmp 5700 system and, for control purposes, on a GeneAmp 7000 system (Applied Biosystems, Foster City, Calif.) starting at 95°C for 10 min, followed by 45 cycles at 95°C, each for 15 s, and then 60°C for 1 min using the TaqMan universal PCR master mix (Applied Biosystems) with the human TaqMan beta-actin detection reagents (Applied Biosystems) for control purposes.

DNA of all three B. burgdorferi genospecies could be detected down to 50 borreliae/PCR for Borrelia afzelii and Borrelia garinii and 100 cells/PCR for B. burgdorferi sensu stricto in B. burgdorferi-spiked urine samples after extraction with DNAzol (Fig. 1). After extraction with the QIAamp and Roche kits, positive signals could be seen only when urine samples were spiked with 500 borreliae (data not shown). Neither 10 urine samples positive by nested PCR after DNAzol extraction nor 12 freshly obtained urine samples from patients with erythema migrans gave a positive signal by Q-PCR, regardless of which extraction procedure was used, although beta-actin was positive in all samples (Fig. 1). The requirements for positive patient samples in earlier experiments were a detection rate of five borreliae/PCR in spiked urine samples. Although optimizing procedures using further primer-probe sequences, primer concentrations, and cycling conditions could improve the outcome of this assay, we assume that unlike nested PCR, one-step Q-PCR as performed by two different primers and probes is not sensitive enough to detect the few borreliae present in urine from patients with erythema migrans.

REFERENCES

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