

P1952 Comparison of two commercial DNA extraction kits for detection of *Brucella* from spiked blood samples and blood culture bottles using two real-time PCR master mixes

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Background: DNA amplification assays have been used for early diagnosis of brucellosis and to reduce risk of laboratory-borne human brucellosis. However, a major limitation of the PCR method is the difficulty to remove inhibitors in samples. The current study was aimed to investigate performance of two DNA extraction kits by using two separate PCR master mixes and to determine appropriate "extraction kit - PCR master mix" combination for diagnosis of *Brucella* from whole blood samples and blood culture bottles.

Materials/methods: Two commercial DNA extraction kits, NORGEN Blood DNA isolation kit (Norgen) and Thermo Scientific GeneJet Whole blood genomic DNA purification kit (Thermo) and two PCR master mixes, QuantiTect multiplex PCR (QuantiTect) and Ampliqon Multiplex TEMPase (Ampliqon) were assessed on 30 blood samples spiked with known concentrations (10^2 - 10^4 cfu / ml) of *Brucella melitensis* ATCC 23456 strain and 10 positive signaling blood cultures. Probes and primers specific to *Brucella* spp., *B. melitensis*, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used in 160 different multiplex real-time PCRs.

Results: PCR with GAPDH probe/primers gave positive results in all of the 120 blood samples. The rate of PCR positivity for *Brucella* spp. was 96.7% for the combination of Norgen -QuantiTect, 93.3% for Thermo -Ampliqon, 93.3% for Thermo-QuantiTect, and 86.7% for Norgen-Ampliqon. *B. melitensis* positivity rates for these combinations were 96.7%, 93.3%, 56.7% and 90%, respectively. In the samples with a bacterial density of 10^2 cfu /ml, *Brucella* spp. detection rates were 80% for Thermo-Ampliqon and Norgen-Ampliqon, and 90% for Thermo-QuantiTect and Norgen-QuantiTect; *B. melitensis* positivity rates were 90%, 70%, 20%, and 90%, respectively. PCR assays with the DNA samples extracted from blood culture bottles using Norgen yielded 80% positivity. However, PCR positivity was only 20% in the DNA samples extracted by Thermo. GAPDH PCRs were also negative in 80% of the samples extracted by Thermo.

Conclusions: Our results revealed that to remove inhibitors and detect even low number of *Brucella* spp./*B. melitensis* in blood samples and blood culture bottles, NORGEN Blood DNA isolation kit can be used with a combination of QuantiTect multiplex PCR or Ampliqon Multiplex TEMPase.