Real-time multiplex PCR assay for detection of *Brucella* spp., *B. abortus*, and *B. melitensis*

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Background: *Brucella* species are facultative intracellular gram-negative bacteria. Brucellosis is an infectious bacterial disease of domestic animals, such as cattle, sheep, goats, camels and dogs, which is caused by brucellae and sometimes results in spontaneous abortions in newly infected animals. Humans are at risk for the disease, especially in areas where the infection in animals has not been brought under control, heat treatment procedures of milk are not routinely applied consumption of raw milk and poor hygienic conditions favor human infection.
Brucellosis was reported in Sudan through both passive and active surveillances in humans and almost all domestic animals, particularly cattle, sheep, goats and camels. Limited wild life studies also revealed the disease in wild reservoirs. This study describes a real-time PCR assay for confirmation of probable *Brucella* isolates from human blood and animal tissues. **Methods:** The assay was designed in a multiplex format that will allow the rapid identification of *Brucella* spp., *B. abortus*, and *B. melitensis* in a single test. A nucleic acid sequences have been targeted for the development of *Brucella* genus-specific PCR assays for the detection of *B. abortus, B. melitensis.* This PCR assay targets the specific integration of IS711 elements within the genome of the respective *Brucella* species. Using similar PCR targets, but in a multiplex format, a real-time triplex assay was developed that permits rapid confirmation of *Brucella spp., B. abortus*, and *B. melitensis* isolates in a single test.Amplification and real-time fluorescence detection was performed on
the iCycler real-time PCR detection system. A sample with a fluorescence signal 30 times greater than the mean standard deviation in all wells over cycles 2 through 10 was considered a positive result, whereas a sample yielding a fluorescence signal less than this threshold value was considered a negative result. To test the specificity of the multiplex assay, an extensive panel of well-characterized *Brucella* and non-*Brucella* strains was assembled and tested. Identification of Brucella strains was performed using standard classification tests including growth characteristics, oxidase activity, urease activity, CO2 requirement, H2S production, dye tolerance, sero-agglutination, and susceptibility to the Tbilisi phage. Non-*Brucella* strains were identified using standard biochemical and immunological procedures and, in some instances, cellular fatty acid analysis and partial 16S rRNA sequencing. The ability to amplify DNA from non-*Brucella* strains was demonstrated using a real-time PCR assay targeting the 16S rRNA gene. **Results:** The sensitivities and specificities of the assay for *Brucella* spp., *B. abortus*, and *B. melitensis* identification were found to be 100 and 100%, 95.0 and 100%, and 100 and 97.0%, respectively. For *Brucella* spp. identification, perfect agreement was found between the phenotypic and genotypic determinations. The PCR method described here uses heat to inactivate the organisms and greatly reduces the risk of laboratory-acquired infection with *Brucella.* Finally, the multiplex format of the assay will reduce reagent cost and staff time required to perform testing for brucellosis.

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