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A quadruplex real-time PCR assays for detection of *Y. pestis, F. tularensis, B. pseudomalle and Brucella*

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Y. pestis, F. tularensis, B. pseudomalle and Brucella are acute infections with high case fatality rates. We describe here the development of a multiplex real-time PCR assay that targets the simultaneous detection of those four pathogens. Species-specific primers and Taqman probes were designed on the highly conserved sequence of each individual pathogen through whole genome sequences alignment, Taqman probes labeled by FAM, Texas Red, JOE and CY5 separately. Using synthetic plasmid DNA as positive control, the multiplexed PCR assay were optimized by evaluating different concentrations of primers and probes to allow for maximum sensitivity and specificity in a tube. The sensitivity of assay were validated to amplify genome DNA of reference strains, as 10×10^3cfu/ml for Y pestis, 14×10^3cfu/ml for F. tularensis, 8×10^3cfu/ml for B. pseudomalle and 6×10^3 cfu/ml for Brucella. The specifity were evaluated against a panel of reference strains as listed Table 1. The Multiplex real-time PCR assay provide a sensitive, reliable and efficient method to detect *Y. pestis, F. tularensis, B. pseudomalle and Brucella* simultaneously, which significantly reduces sample processing time, amount of labor required, and consumable costs while yielding an increase in diagnostic power and a high sensitivity and specificity. This method has good prospects of application for disease prevention.

Biography

Yu Yang graduated from China CDC with a PhD in 2006 for her work on the reversed genetics of Sendai virus, and then Post-doctoral experience at University of Buffalo, working on developing transposon mutagenesis system in Borrelia burgdorferi. In 2008, she moved to the Chinese Academy of Inspection and Quarantine (CAIQ) of AQSIQ undertakes work on predicting, diagnostics, prevention and control for infectious disease. She has led a number of projects in this area ever since, developed detection methods against a lot of pathogens such as *Zika virus*, *MERS-CoV, Rickettsia, C. burnetii, E. coli* O104, *Y. pestis, F. tularensis, B. pseudomalle and Brucella*. Her research interests include the development of molecular and immune diagnosis by real- time PCR, Bio-plex, immune colloidal gold technique, and currently involved in developing novel magnetic nano-labels methods and metagenomics technique for pathogen detection. She has published extensively in this area and related disciplines, and received several research awards, with 32 technology patents.

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