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**A real-time PCR array for hierarchal identification of environmental and human pathogenic *Francisella***

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A robust, rapid and flexible real-time PCR assay for hierarchal genetic typing of clinical and environmental isolates of *Francisella* is presented. Typing markers were found by multiple genome and gene comparisons. Twenty-three canonical single nucleotide polymorphisms and 11 canonical insertion-deletion mutations were selected to serve as phylogenetic guidelines for classification ranging from genus to isolate level. Specificity was assessed using 62 *Francisella* isolates of a diverse genetic and geographical origin. The final assay is in a 96-well plate real-time PCR format and was successfully used for typing of 14 *F. tularensis* subsp. *holarctica* isolates from tularemia patients in Sweden year 2008 and five genetically more diverse *Francisella* isolates of a global origin. The detection limit of the 96-well assay was 100 pg. When applied to human ulcer specimens for direct pathogen detection, the results were incomplete. Nevertheless, a selected subset of markers could be analyzed to expose fine resolution among *F. tularensis* subsp. *holarctica* causing infection in the patients. In contrast to other real-time PCR assays for *Francisella* that are typically designed for specific detection of a species, subspecies, or strain; this type of assay can easily be tailored to match a preferred phylogenetic and geographical resolution level.