Eur J Clin Microbiol Infect Dis 2009;28:1363-1368

Real-Time PCR Strategy and Detection of Bacterial Agents of Lymphadenitis

E. Angelakis, V. Roux, D. Raoult, J-M Rolain

Background: Lymph node enlargement is a common medical problem that is usually caused by bacterial, viral, fungal and protozoal agents. *B. henselae*, the agent of cat scratch disease (CSD), appears to be the most common organism responsible for lymphadenopathy, both in adults and children. However, before its discovery and the use of PCR, mycobacteria were the most common infectious agents causing lymphadenopathy, and staphylococci and group A streptococci were the main causes of acute adenitis. Other rare or under-reported causative agents have been linked to infectious lymphadenophy, including *Francisella tularensis*, the agent of tularaemia, and *Tropheryma whipplei*, the agent of Whipple's disease. The problem with lymph node enlargement is that clinical symptoms are often similar or non-specific so that several etiological agents could be responsible for the disease. For example, lymphadenopathy due to *B. henselae* may resemble that due to *Francisella tularensis.* Moreover, the most common etiological agents are fastidious bacteria that are not easily or rarely isolated by conventional or specialized cell culture systems. Thus, molecular methods are considered to be the gold standard methods for the detection of these fastidious bacteria from lymph node biopsy specimens. However, because any of these bacteria may be responsible for the disease, a single molecular approach for the detection of these bacteria is difficult and clinical microbiology laboratories commonly used the universal 16 S rDNA gene amplification and sequencing for the identification of the causative agent. Nevertheless, this method has been reported to lack sensitivity, especially for the detection of *B. henselae* and concomitant diseases with other bacteria such as *M. tuberculosis* may be missed.

The aim of this study was to compare 16 S rRNA gene amplification and sequencing with a systematic real-time PCR assay screening strategy that includes all common known pathogens recovered from lymph node biopsy specimens. Lymph node biopsy samples sent to our laboratory from January 2007 to December 2008 were tested in the study. Lymph nodes were screened for the presence of any bacteria by PCR amplification and sequencing targeting the 16 S rRNA gene and also by a specific real-time PCR strategy that includes *Bartonella henselae*, mycobacteria, *Francisella tularensis*, and *Tropheryma whipplei*. By testing 491 lymph nodes, we found that the sensitivity of our specific real-time PCR assay strategy was significantly higher than 16 S rRNA PCR amplification and sequencing for the detection of *Bartonella henselae* (142 vs 98; $p < 10^{-4}$), *Francisella tularensis* (16 vs 10, $p < 10^{-4}$), and mycobacteria (8 versus 3, $p < 10^{-4}$). None of the samples was positive for *Tropheryma whipplei*. Our study demonstrates the usefulness and specificity of a systematic real-time PCR strategy for molecular analysis of lymph node biopsy specimens and the higher sensitivity compared with standard 16 S rRNA gene amplification and sequencing.