ABSTRACT

*Pneumocystis carinii* is an opportunistic pathogen, which often causes fatal pneumonia in patients under immunosuppressed or immune deficient conditions due to AIDS, cancer chemotherapy, or immunosuppressive therapy for organ transplantation.

Different techniques of microscopy and a nested polymerase chain reaction (PCR) are widely used for detection of this opportunistic fungus. But these methods are less sensitive and time-consuming. We focused our attention on the level of specific DNA by a quantitative PCR technique. This procedure has the advantage of greater precision and more objectivity.

In this report we describe a real-time PCR assay suitable for use with the LightCycler system. We were successful in implementation and optimization of quantitative real-time PCR for *Pneumocystis carinii*. We have reached sensitivity 1000 copies of DNA *Pneumocystis carinii*/ml. We have set a limit of detection to be 50 copies of DNA *Pneumocystis carinii*/ml.

Real-time PCR procedure was optimized and validated in laboratory of molecular biology of Department of clinical biochemistry and diagnostics of Fakultní Nemocnice in Hradec Králové. A standard routine was established to be utilized in general practice.