Application of Real-Time PCR to the Diagnosis of Invasive Fungal Infection

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Abstract

The management of invasive fungal infections has been hampered by the inability to make a diagnosis at an early stage of the disease. Molecular diagnosis by PCR appears very promising since fungal DNA can be detected in the blood of infected patients earlier than when using conventional methods. Recently, interest in the diagnosis of invasive fungal infections by real-time PCR has increased. Real-time methods also have quantitative properties and are useful both for initial diagnosis and to assess the response to treatment. Many recent studies have combined serological tests with measurement of fungal DNA by using real-time PCR. Real-time PCR helps early diagnosis and arrangement of treatment protocols for patients with high risk of fungal infection. Here real-time PCR methods for diagnosis of invasive fungal infections are described and discussed.

Introduction

Rapid tests with high specificity and sensitivity are needed for early diagnosis of invasive fungal infections, which have non-specific clinical signs. The major groups of organisms involved are the *Aspergillus* and *Candida* spp. Blood culture and serology are the conventional methods used for diagnosis of these fungi but they have limited sensitivity, specificity and do not give a rapid indication of current infection. Consequently, ELISAs for detection of antigens (*e.g.* galactomannan and glucan) and nucleic acid detection techniques have come into use for the differential diagnosis and follow-up of fungal infections.

Tests for fungal infection are especially important for transplant recipients and patients with hematological malignancies, who have high mortality and morbidity ratios. New advances in cancer treatment and intense supportive treatment provide longer life for cancer patients but morbidity and mortality ratios increase due to invasive fungal infections. Fungal infection is now the cause of death in about half of the patients with acute leukemia. The most important factor for successful antifungal treatment in cancer patients, is to make a positive diagnosis but this may be difficult. Atypical clinical findings, difficulty in taking samples and insufficient diagnostic methods are basic problems. Although certain clinical signs and symptoms found in appropriate patient groups might indicate fungal infection the correlations are not strong. Different fungal and bacterial infections and even non-infectious conditions may cause the same clinical appearance. For many years culture has been the gold standard for diagnosis of fungal infection but taking samples for culture is problematic. Deep tissue biopsies for culture from patients with thrombocytopenia or pulmonary infiltration are particularly difficult to take. Failure to make an accurate diagnosis may prevent the administration of optimal treatment (Anaissie, 1992; Einsele *et al*., 1997; Denning, 1998; Van Burik *et al*., 1998).

Although the use of NASBA for detection of 18S rRNA is promising, most of the nucleic acid-based methods use PCR for detection of fungal DNA and the results have been encouraging (Kami *et al*., 2001; Loeffler *et al*., 2001). Often the primers are directed to conserved genes

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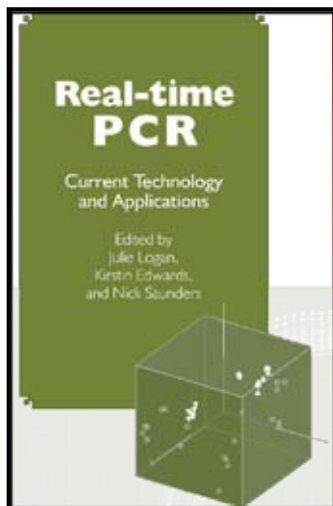**c. 262 pp., January 2009**
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Real-time PCR (RT-PCR) technology is highly flexible and many alternative instruments and fluorescent probe systems have been developed recently. The decreased hands-on time, increased reliability and improved quantitative accuracy of RT PCR methods have contributed to the adoption of RT PCR for a wide range of new applications.

This essential manual presents a comprehensive guide to the most up-to-date technologies and applications as well as providing an overview of the theory of this increasingly important technique. Renowned experts in the field describe and discuss the latest PCR platforms, fluorescent chemistries, validation software, data analysis, and internal and external controls. This timely and authoritative volume also discusses a wide range of RT-PCR applications including: clinical diagnostics, biodefense, RNA expression studies, validation of array data, mutation detection, food authenticity and legislation, NASBA, molecular halotyping, and much more.

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