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Rapid PCR-Based Detection of *Streptococcus pneumoniae* DNA in Cerebrospinal Fluid

The reports by Cherin et al. (2) and Garcia et al. (3) document the potential advantages of PCR-based assays for the detection of *Streptococcus pneumoniae* DNA in cerebrospinal fluid (CSF) and lung tissue, particularly when culture is compromised by antibiotic therapy. In both instances the (different) PCR-enzyme immunoassays (PCR-EIAs) showed excellent sensitivity, detecting approximately 3 CFU and 2 to 27 CFU, respectively.

These studies also highlight a dilemma in applying PCR-based assays to acute clinical problems. In the study by Garcia et al. (3), PCR-EIA was more sensitive than latex agglutination for capsular polysaccharide antigen (LA). This advantage was more than offset by the speed of the LA (30 min) compared with that of the PCR-EIA (10 h). It was suggested that PCR-EIA be reserved for research purposes. However, significant and costly differences in antibiotic management could attend rapid demonstration of the presence or absence of *S. pneumoniae* DNA in a normally sterile site, particularly in cases of pneumonia.

We have used a LightCycler (Idaho Technology Inc., Idaho Falls, Idaho), which performs real-time PCR, combining rapid cycling with fluorescent measurement in glass reaction capillaries (1). We targeted the pneumolysin gene of *S. pneumoniae* using an asymmetric PCR approach, the forward primer being labelled with Cy5 (cyanine 5) and a sequence-specific fluorescein-labelled oligonucleotide probe being incorporated into the reaction mixture, allowing identification of the PCR product by fluorescent resonance energy transfer (4) between the fluorescein (donor) and the cyanine 5 (acceptor) when these fluorophores became adjacent due to hybridization of the probe. Apparently negative samples were retested after "spiking," to allow the detection of inhibitors in the sample. The assay required 2 μl of sample per reaction (test and spiked control). The sensitivity was found to be 15 CFU.

This assay was then used on 12 CSF samples; the results are shown in Table 1. Two of the culture-negative, LA-negative, PCR-negative samples were from patients with viral meningitis, a third was from a patient with proven meningococcal meningitis, and the fourth sample was a follow-up specimen from an *S. pneumoniae* PCR-positive patient after 2 weeks of intravenous (i.v.) cefotaxime therapy and clinical improvement. Amplification occurred in all four PCR-negative samples after spiking, demonstrating the absence of inhibitors. The clinical features of the three culture-negative, LA-negative, PCR-positive patients were compatible with pneumococcal infection, and in all cases PCR tests for *Neisseria meningitidis* DNA with blood and CSF were negative. One patient had a concomitant acute mastoiditis. A second (adult) patient presented with low-grade meningitis and internal hydrocephalus 1 month after amoxicillin treatment for lobar pneumonia. CSF obtained at the time of the operation was culture and LA negative but PCR positive. This patient responded to high-dose i.v. cefotaxime. The third patient, aged 54 years, had concomitant meningitis and pneumonia.

TABLE 1. Results of real-time PCR for detection of *S. pneumoniae* DNA in CSF specimens from patients with clinical meningitis

Conventional test result	No. of specimens	No. positive by real-time PCR
Culture positive, LA positive	2	2
Culture positive, LA negative	1	1
Culture negative, LA positive	2	2
Culture negative, LA negative[a]	7	3

[a] For details, see text.

Our assay minimizes cross-contamination (it is a closed-tube system) yet has a sensitivity comparable to that of nested PCR or PCR-EIA. The amplicon is identified with a sequence-specific hybridization probe. Our PCR test requires only 4 μl of sample. The assay run time is 40 min. To this must be added the time needed for extraction of DNA from the submitted material. In this study, the samples were simply boiled for 15 min prior to PCR testing.

Real-time PCR assays offer the prospect of both the sensitivity and speed required to challenge, and even supplant, conventional nonculture methods for the diagnosis of *S. pneumoniae* infection of normally sterile sites.

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