

Quantitation of *Pseudomonas aeruginosa* in wound biopsy samples: from bacterial culture to rapid 'real-time' polymerase chain reaction

ABSTRACT

The three methods showed a good concordance with the culture results. Conventional PCR was at least 100 times less sensitive than bacterial culture and had a low dynamic range (2 logs). With a lower detection limit of 103 CFU/g tissue, ELISA-PCR was ten times more sensitive than conventional PCR. The dynamic range, however, did not increase. ELISA-PCR is very time consuming (8 h). The RTD-PCR produced a linear quantitative detection range of 7 logs with a lower detection limit of 103 CFU/g tissue. More important, however, was that the time from sample collection to result was less than 1 h. Two biopsy specimens scored significantly higher in ELISA-PCR and RTD-PCR than in bacterial culture. This could indicate that DNA from dead bacteria was amplified. One out of ten culture positive biopsy samples was found negative by all PCR-based methods. Topical antimicrobial agents possibly inhibited PCR. These results show that RTD-PCR has potential for the rapid quantitative detection of pathogens in critical care patients, enabling early and individualized treatment. Further study is required to assess the reliability of this new technology, and its impact on patient outcome and hospital costs.

INTRODUCTION

Introduction Although effective topical antimicrobial chemotherapy and early excision of burn wounds have significantly reduced the occurrence of invasive burn wound infections, sepsis is still a major problem. The risk of septicaemia increases in proportion to the degree of cutaneous infection. Many investigators have reported that quantitative biopsy culture was the best method for early detection of sepsis. Heininger et al stressed that only 4-12% of blood cultures is found positive. Conversely, McManus et al reported in 1987 that high tissue counts did not necessarily indicate invasion, and that the principal value of quantitative biopsy culture was the demonstration of the predominant burn wound flora. Even so, when sepsis ensues, while awaiting the results of blood cultures, a knowledge of the organisms that colonize a burn wound can facilitate prompt and appropriate antibiotic treatment that is based on the expected sensitivity of the identified germs, rather than initiating a purely empirical therapy. There is need for qualitative and quantitative tests that are more rapid than bacterial culture. We decided to develop such a test for the rapid detection and quantitation of *Pseudomonas aeruginosa* in burn wound biopsy samples. This bacterium is ubiquitous, is inherently resistant to common antibiotics, and therefore is one of the most problematic pathogens in modern hospitals. Burn wound patients, mechanically ventilated patients and cystic fibrosis patients are particularly susceptible to *P. aeruginosa* infection. Biopsy samples are, with respect to homogenization and DNA extraction, very tough clinical samples and thus would be an excellent test case for the applicability of the method on direct clinical samples. In 1997 our group developed a PCR test for the direct detection and identification of *P. aeruginosa* in clinical samples that is based on the amplification of the outer membrane lipoprotein gene *oprL*. Since then we developed several quantitative variants of this test, exploiting the technology available at the time. In the first instance, we amplified the *oprL* gene by means of conventional PCR and visualized the PCR product by ethidium bromide (EtBr) staining of agarose gels. The intensity of the fluorescence produced by EtBr was quantified. Second, we developed an ELISA-mediated PCR in

order to quantify the amplified oprL gene. PCR products were labelled with digoxigenin during the amplification process and quantitatively detected by absorbance reading in microtitre plates. Finally, we exploited the recently developed 'real-time' quantitative PCR technology. We opted for the LightCycler™ system (Roche Diagnostics, Brussels, Belgium) because it features rapid capillary tube resistive thermal cycling, reducing the amplification time dramatically. Two adjacent hybridization probes, labelled with different fluorescent dyes, are used to monitor the appearance of PCR product. The emitted light signal is proportional to the amount of specific DNA product available for hybridization, and thus increases every cycle. The probes were designed to be complementary to a conserved region of the oprL gene, as determined by sequence analysis of the oprL gene of 85 nonrelated clinical *P. aeruginosa* isolates. In the present report we compare the assay performance of the above-mentioned methods, in terms of practicability, to bacterial culture. For this purpose three types of samples were assayed: serial *P. aeruginosa* dilutions, uninfected skin spiked with *P. aeruginosa* and 21 burn wound biopsy samples. All methods were useful, but only Light-Cycler™ RTD-PCR allowed rapid quantitative detection of *P. aeruginosa* in skin biopsies with an adequate detection limit and a wide log-linear range.

CONCLUSION

Up until now, RTD-PCR has been applied for the detection of food-borne pathogens, cancer, genetic diseases and infectious diseases. Although a limited number of clinical specimens were tested, the present results indicate that RTD-PCR, and more specifically LightCycler™ technology, has potential for quantitative applications in the clinical laboratory. In particular, it has applications for the critical care population, at the point of care, and it is important that the test is subjected to further optimization and evaluation. Early infection diagnosis remains a difficult problem for patients with burn wounds or cystic fibrosis, and for critical care patients in general. Prognosis and survival are often dependent on an early, individualized treatment. Automated extraction of DNA from a variety of clinical samples (blood, expectorations, urine, etc.) and subsequent rapid, online, quantitative detection of pathogens (*P. aeruginosa*, *Staphylococcus aureus*, *Haemophilus influenzae*, among others) by RTD-PCR is now possible, allowing early therapeutic decisions to be made. Multiple colour detection will open the door to multiplex RTD-PCR. Further studies are necessary to assess the impact of rapid RTD-PCR on patient outcome and hospital costs.