

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

## ABSTRACT

Among the three methods used, one was able to detect pathogens more quickly than those used for bacterial culture, with a high degree of precision.

## INTRODUCTION

Introduction (1) the use of *Pseudomonas aeruginosa* in wound biopsy samples (e.g., wound tissue biopsies); (2) the identification of this isolates as *Pseudomonas aeruginosa* and (3) the characterization of the different strains of *Pseudomonas aeruginosa* isolated from wound samples.

Methods: (1) *Pseudomonas aeruginosa* were isolated from wound biopsy samples collected from patients with acute myocardial infarction (AMI). *Pseudomonas aeruginosa* were isolated from wound biopsy samples from patients with chronic myocardial infarction (CMI). The isolation of *Pseudomonas aeruginosa* was performed using Our objective was to devise a test that could quickly identify and quantify *Pseudomonas aeruginosa* in burn wound biopsy samples. As the bacterium is highly prevalent, resistant to common antibiotics, and therefore one of the most challenging pathogens in hospitals today. This method would be ideal for biopsy samples that are clinically difficult to homogenize and extract DNA from, making them an excellent test case for the applicability of this method on direct clinical samples (hybridity above 90% ratio and Peptium year-round patient relationship) and the resistance to P Our team developed a PCR test in 1997 that employs the outer membrane lipoprotein gene *oprL* to directly detect and identify *P aeruginosa* in clinical samples. Since then, we have developed several quantitative variants of this test, utilizing the technology available at the time. Initially, we utilized the conventional PCR method to increase the *oprL* gene and then stained agarose gels with ethidium bromide (EtBr) to visualize the resulting RNA. The quantification of the fluorescence produced by EtBa was done to measure the intensity of its fluorescence. To measure the amplified *oprL* gene, we used an ELISA-mediated PCR. During the amplification process, amplifiers were labelled with digoxigenin and their products were quantified by measuring the absorbance of the product in microtitre plates. In the end, we made use of the newly discovered quantitative PCR technology. We chose the LightCycler<sup>TM</sup> system (Roche Diagnostics, Brussels, Belgium) because it features fast capillary tube resistive thermal cycling, which dramatically reduces the amplification time. The system includes two adjacent hybridization probes that are labeled with different fluorescent dyes to track the appearance of RNA and DNA product. Each cycle increases proportionally with the amount of specific DNA produced for hybridisation, as determined by sequence analysis of 85 nonrelated clinical *P aeruginosa*. The present report scrutinizes the efficacy of the aforementioned methods when applied to bacterial culture. We tested three types of samples, including serial *P aeruginosa* dilutions, uninfected skin spiked with *P A*, and 21 burn wound biopsy samples. All methods were effective, but only Light-Cycler<sup>TM</sup> RTD-PCR provided fast quantitative detection of *P ApoE* in skin biopsies with an adequate detection limit and broader log-linear range.

## CONCLUSION

RTD-PCR has been utilized to detect food-borne pathogens, cancer, genetic diseases, and infectious diseases until now. Despite the limited number of clinical samples tested, the current findings suggest that RTD-PCR and LightCycler™ technology have potential quantitative applications in the clinical laboratory. This includes specific applications for the critical care population, at the point of care, and critical condition diagnosis. Early infection diagnosis is still a challenging task for patients due to early, personalized treatment. Rapid online detection of pathogens like *Staphylococcus aureus* and *Haemophilus influenzae* can be used to make early therapeutic decisions. Additional studies are needed to determine whether multiplex Rt-oxioxin exposure and reaction conditions.