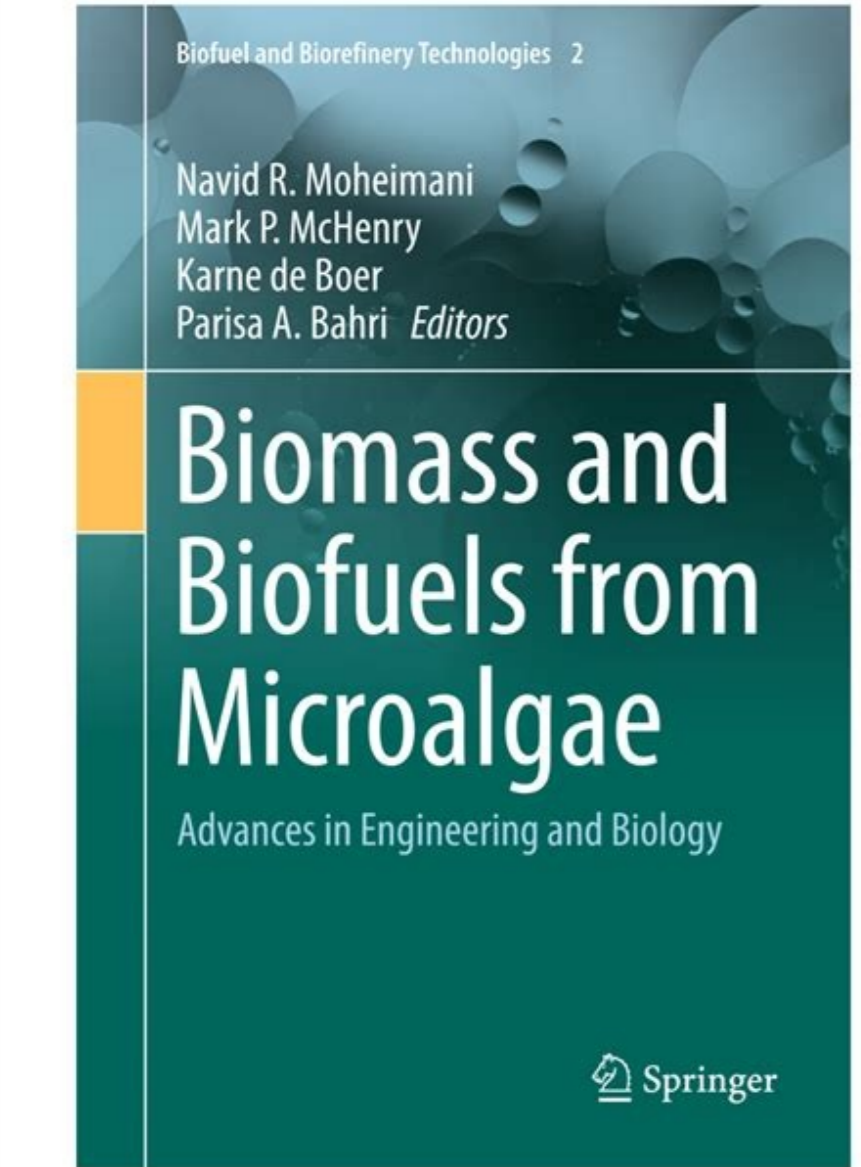


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Molecular Biology  
Validation of a Real-Time PCR (qPCR) Technique for Detection of *Mycobacterium ulcerans* in Clinical and Environmental Samples

**Abstract**  
Introduction: Buruli ulcer (BU) is a serious skin disease caused by *Mycobacterium ulcerans*. According to WHO, 75% of BU cases should be confirmed by the PCR-based gene amplification. The objective of this study is to validate a real-time PCR for the detection of *M. ulcerans* in clinical and environmental samples.

**Methods**: A total of 75 clinical samples, 33 *M. ulcerans* strains and 15 environmental samples were tested by Ziehl-Neelsen staining technique, conventional PCR, real time qPCR and culture. The proportion of positive cases of *M. ulcerans* infection between the different tests were compared by the Chi-square test. The difference was statistically significant for a P-value < 0.05.

**Results**: Out of 12482 samples were cultured positive (41.25%) to *M. ulcerans*. 4180/29 staining were positive (51.28%) to AF at the microscopy, 5590/39 (79%) and 8400 samples (97%) were positive to the IS2474 insertion sequence from conventional PCR and qPCR respectively. Both PCR techniques showed positivity rates significantly higher than microscopy and culture (P<0.002). However, no significant difference of positive rate was observed between the two types of PCR (P=0.56). In contrast, the detection limit for the real time qPCR was lower (120 copies/μl) compared to conventional PCR. Only qPCR was able to detect IS2474 in 2015 environmental samples (13.3%).

**Conclusion**: This study showed the high sensitivity of the real time PCR for the detection of *Mycobacterium ulcerans* in clinical and environmental samples.

**Keywords**: Buruli ulcer; *Mycobacterium ulcerans*; Classical PCR; real time PCR; Ziehl-Neelsen; Culture

**Introduction**  
Buruli ulcer (BU) is an emerging infectious disease caused by *Mycobacterium ulcerans*. This disease is characterized by extensive destruction of the skin and soft tissue with formation of ulcers and necrosis. The necrotizing and ulcerative character is induced by a diffuse chronic granulomatous response, called granuloma, which is produced by *M. ulcerans* virulence genes. Buruli ulcer cases are reported in more than 33 countries, mainly tropical or subtropical, but are also present in temperate climates such as Japan and southern Australia [1]. According to WHO, between 1,000 and 1,200 cases of Buruli ulcer are reported annually worldwide with more than 8,000 cases occurring in Sub-Saharan Africa. The higher number of reported cases are found in West African continent with 3.06 cases/km with about 2000 cases per year. France and Ghana 1000 cases per year [2].

The spreading of Buruli ulcer cases is based on laboratory confirmation using WHO recommended tests for the diagnosis of the disease, including direct examination of smears for acid fast bacilli (AFB) in smears culture and gene amplification (PCR) targeting the insertion sequence IS2474. According to WHO, 75% of BU cases should be confirmed by the PCR-based gene amplification technique [3]. The development of the conventional PCR technique targeting IS2474 sequence, as insertion of more than 200 copies present in *M. ulcerans* genome has become a rapid technique for confirming cases of Buruli ulcer. This technique was also used to analyze environmental samples and to detect IS2474, thus allowing the probable presence of *M. ulcerans* in water samples [2], aquatic insects [3]. Although this technique is sensitive and specific for *M. ulcerans* detection in clinical samples, its application on environmental samples remains difficult and non-specific due to the presence of PCR inhibitors. In order to increase specificity, confidence in

the results of PCR and facilitate rapid analysis of clinical and environmental samples, Yap et al. have developed two real-time multiplex techniques targeting the IS2474 insertion sequence [4].

In Togo, the availability of a national reference laboratory using the conventional PCR technique makes it possible to confirm each year about 30 to 40 new cases in the endemic districts of Zio and Yoto in the maritime region [5]. However, this technique requires access time and method work due to the gel preparation and DNA separation in electrophoresis. It is therefore important that this technique should be the most sensitive and specific to ensure a reliable diagnosis test. This study aims to validate a real-time PCR for the detection of *M. ulcerans* in clinical and environmental samples through IS2474 sequence detection in order to improve the level of the sensitivity of the PCR in the diagnosis of Buruli ulcer.

**Materials and Methods**  
**Biological material**  
Strains of *M. ulcerans* (Strains of *M. ulcerans* were obtained from the Reference Laboratory of Microbiology of Brest LERMU (ERMU 1).

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