The spirochete *Treponema pallidum* subsp *pallidum* is the etiologic agent of syphilis, a sexually transmitted infection (STI), a disease with multiple stages. Syphilis diagnosis is based on clinical signs associated with serodiagnostic tests. The molecular subtyping technique is a robust tool to investigate diversity and epidemiology of *T. pallidum*. This study aimed to characterize one isolate of *T. pallidum* in a swab specimen in Dourados, Brazil. The primary syphilis was confirmed by a combination of clinical symptoms and serological results, with rapid syphilis test (Alere, Republic of Korea) and the Venereal Disease Research Laboratory test (Wama Diagnóstica Belgium). DNA was extracted from swab obtained from syphilis lesion by QIAamp DNA Blood kit (Quiagen, USA). For the detection of treponemal DNA in swab sample, a traditional PCR targeting of polA gene of *T. pallidum* was performed the subtyping was determined by Enhanced Centers for Disease Control and Prevention typing (ECDCT). This method is based on the 1) number determination of 60 bp repeats in the *arp* gene; 2) RFLP (*Restriction fragment length polymorphism*) of *tp* gene of subfamily II (*tpr EGJ*); 3) analysis of 84 pb region of *tp0548* gene. The *tp0548* gene sequence showed total equivalence with the “g” type sequence, which is the most European prevalent type. We identified for the first time in Brazil, the partial strain-type 14 X/g in primary syphilis ulcers. The results indicate that the molecular typing from the lesion effectively detected *T. pallidum* subtype, becoming a useful tool for epidemiological investigation. The capability to identify syphilis strain types in infected patients may have relevant clinical implications, once it would help to identify patients with potential neurosyphilis risk, as they commonly infected with the 14d/f subtype for having a more neuro invasive risk. Subtyping methods are efficient in identifying and discriminating re-infections, as well as geographical identification. This method demonstrate the relevance of studying molecular epidemiology for better effective prevention and control of syphilis transmission.

**Keywords**, PCR, Syphilis, Molecular typing

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