MOLECULAR DIAGNOSIS OF SYPHILIS: APPLICATION OF ANCIENT DNA PROTOCOLS FOR THE DETECTION OF *Treponema pallidum* SUBSP. *pallidum* IN SEROLOGICAL SAMPLES

LORRAYNE SAMILLE SANTOS DE BRITO[1,2], ALENA MAYO IÑIGUEZ[1].

[1]LABORATÓRIO DE PARASITOLOGIA INTEGRATIVA E PALEOPARASITOLOGIA – INSTITUTO OSWALDO CRUZ/FIOCRUZ, RIO DE JANEIRO

[2]PROGRAMA DE PÓS-GRADUAÇÃO EM BIOLOGIA PARASITÁRIA - INSTITUTO OSWALDO CRUZ/FIOCRUZ, RIO DE JANEIRO

Syphilis is an ancient, congenital, and sexually transmitted infectious disease that remains a major public health challenge, with an increasing incidence in Brazil and worldwide. Although traditional diagnostic methods, such as serological tests like VDRL and TPHA, are widely employed, they have limitations, mainly in the early stages of infection, due to a high rate of false positives. The detection of *Treponema pallidum* subsp. *pallidum*, the causative bacterium of syphilis, is hindered by the low recovery of the spirochetes in relation to the different phase of disease. However, some biological samples with minimal bacterial concentration, such as serum, could be an alternative source for highly sensitive molecular diagnosis. To address this issue, an ancient DNA (aDNA) protocol was applied to optimize the molecular diagnosis of syphilis from serological samples. DNA extraction and molecular reconstruction aDNA protocols were performed, followed by PCR and Sanger sequencing for detecting *T. pallidum* subsp. *pallidum* in 18 patients from healthcare units specializing in sexually transmitted infections, Rio de Janeiro. The selected serum samples showed different positivity titers for VDRL test of 1:2, 1:4, 1:8 and non-reactive as negative controls. Enzymatic amplifications were conducted using *tpp*15, *tpp*47, and *pol*A *T. pallidum* markers. PCR results revealed positivity in 13/18 patients and preliminary nucleotide sequencing analysis confirmed *T. pallidum.* subsp. *pallidum* identification in 10/13. The results demonstrated the effectiveness of the applied aDNA methodology for precise bacterial identification in serological samples. Despite the challenge of molecular detection on serum, the aDNA approach employed allowed a confirmatory specific diagnosis of syphilis. Integrating molecular techniques, including aDNA-specific protocols, may enhance the diagnostic accuracy and help with disease control strategies, contributing to improved public health outcomes in Brazil and globally.

SUPPORT: Capes; Faperj; CNPq; LPIP-IOC/FIOCRUZ

KEYWORDS: Syphilis Diagnosis, *Treponema pallidum*, Molecular Detection