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Use of brief culture on Lowenstein–Jensen media as pre-polymerase chain reaction (Pre-PCR) to detect *Mycobacterium*

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ABSTRACT

Background and aim: Mycobacterium tuberculosis (MTB), the causative agent of tuberculosis (TB), does not retain any bacteriological stain due to the high lipid content in its wall. Classic methods like acid-fast staining or Ziehl–Neelsen staining are used for the diagnosis. Nucleic acid amplification techniques, such as polymerase chain reaction (PCR), are very useful in the rapid diagnosis of infections by MTB, but the sensitivity of PCR is considerably lower because of the presence of inhibitory substances in tissues. The aim of this study is to improve the sensitivity of PCR in inhibiting tissue samples.

Method and material: Thirty-six lymph nodes isolated from inoculated guinea pigs with BCG vaccine were used. After grinding and suspending samples in sterile distilled water, they were cultured on Lowenstein–Jensen media. DNA extraction was done after 5 days by cetyl-trimethylammonium bromide (CTAB) method. Finally, PCR was done for each extracted DNA.

Result: This study began with 40 guinea pigs; 4 died before the fourth week. Thirty-six lymph nodes were sent to PRIC lab; all 36 samples were positive in culture and ZN stain. All specimens were assessed by two methods: PCR before and after 5 days of culture. 27.8% (10 samples) and 69.4% (25 samples) were positive PCR before and after brief-culture, respectively.

Conclusion: In summary, the aim of this study was to demonstrate the importance of optimal DNA extraction and brief-culture for elevating the efficacy and accuracy of PCR for the rapid detection of lymph node TB.

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