

Effects of temperature and media on preservation and recovery of *Mycobacterium tuberculosis* strains

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ABSTRACT

The preservation and recovery of *Mycobacterium tuberculosis* (MTB) strains isolated from clinical specimens is an important stage in the understanding of issues pertaining to tuberculosis control. Well preserved strains provide readily available and cost effective material that is useful in facilitating studies that address pertinent issues.

This study was done to determine optimum temperature(s), suitable media and conditions for recovery that sustain high survival rate of MTB strains for five months at Center for Respiratory Diseases Research KEMRI laboratory.

A total of 97 randomly selected strains were aliquoted into two aliquots of the suspension media containing each; 1% gelatin buffered pH 6.8 (gelatin), 15% aqueous solution of lactose pH 5.0 (lactose), 5% sodium glutamate + glycerol (SG), trypticase soy broth + glycerol (TSB), phosphate buffered saline pH 7.2 (PBS), Middlebrook 7H9 (7H9), skimmed milk (SM), OADC-enriched Middlebrook 7H9 + 5% glycerol (OADC-7H9) and sterilized distilled water (SDW).

The aliquots were preserved using two approaches each utilizing four temperatures: room temperature (RT), 4°C, -20°C, and -80°C. In the first approach, strains were preserved directly to the respective temperature. In the second approach a systematic step method starting from RT to 4°C, to -20°C, and to -80°C with one hour interval, was utilized. After each subsequent month for five months, strains were thawed by systematic approach starting from -80°C to -20°C to 4°C to RT. For the second approach strains were directly thawed after five months preservation. The thawed strains by both approaches were subcultured on Lowenstein Jensen, incubated at 37°C for four weeks and -growth was graded according to the standard method.

Cross-tabulation of grading of growth was performed to determine interrelation between temperature and media using 7H9 medium as the standard. Significant differences within and between the grading of each suspension media were determined by using the Kendall's coefficient of concordance. A $p < 0.05$ was regarded as significant.

In the first approach at -80°C OADC-7H9 had the highest yield while SDW had the lowest yield ($p < 0.05$). At -20°C SG had highest yield while SDW had the lowest yield ($p < 0.05$). At 4°C SG had highest yield while PBS had the lowest yield ($p < 0.05$). At RT SM had the highest yield while TSB had the lowest grading ($p < 0.05$).

In the second approach, at -80°C , 5% SG had the highest yield while SDW had the lowest yield $p < 0.05$. At -20°C , 5% SG had highest yield while SDW had the lowest yield $p < 0.05$. At 4°C , 5% SG had highest yield while PBS had the lowest yield ($p < 0.05$). At RT SM had the highest yield while TSB had the lowest grading ($p < 0.05$).

When approaches one and two were compared for temperature and media, there was no statistically significant difference ($p < 0.05$).

This study showed that optimum recovery of MTB strains was mainly dependant on a combination of appropriate temperature and suitable media. The media that was consistent with high recovery was sodium glutamate while the best preservation temperature was -80°C . More studies are required to determine the effect of preservation using approach one and recovery using approach two and vice versa In addition extension of the preservation duration beyond five months should be further elucidated.