

## Comparison of Molecular Methods for Detection of Pyrazinamide Resistant *Mycobacterium tuberculosis* Isolates

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**Background & Objectives:** Pyrazinamide, is an one of four first-line TB drugs. In these study molecular methods has been designed for detection of resistant isolates.

**Methods:** From 80 clinical isolates of *Mycobacterium tuberculosis*, 39 isolates including 12 resistant 5, susceptible and 22 strains with unknown resistance phenotype to Pyrazinamid were selected for evaluation of possible mutations. ASP (Allele Specific PCR ) and Semi Nested-ASP (SnASP) Methods were used for detection of wild state, any mutations in nucleotides of 374 and 359 of pncA gene. PCR-RFLP methods was designed by SacII enzyme (for detection of mutation in nucleotides 359). Results of phenotypic culture methods and sequencing methods were used as gold standard.

**Results:** As a result from ASP methods, from 12 resistant isolates to Pyrazinamide, 6 mutant and 6 non-mutant strains were determined. Five Susceptible strains by all molecular Methods were revealed lack of any mutation. From 22 strains with unknown phenotype to Pyrazinamide, 3 mutant strains and 19 non-mutant strains were determined. Sequencing methods was proved the results. Enzyme SacII (specific nucleotide 359) in PCR-RFLP methods was not able to detect resistant strains. Sequencing results demonstrated that the mutant strains in this study were lack of mutations in 359. Therefore, this enzyme showed alow operational value.

**Conclusion:** Semi Nested-ASP specific primers designed for the methods was able to detect susceptible strains and recommends as a rapid detection Methods for routine use.

**Keywords:** *Mycobacterium tuberculosis*; PCR-RFLP; Semi Nested-ASP; Pyrazinamide