IVD_14 - Development of a complete point-of-care diagnostic solution (DNA extraction + qPCR) for detecting resistance to antibiotics used in tuberculosis treatment

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Introduction: Caused by the pathogen Mycobacterium tuberculosis, tuberculosis is considered by WHO as a disease of highly aggravating impact. Although there are effective antibiotics against the bacillus, several situations of resistance and multidrug resistance to them have already been reported. Single nucleotide polymorphisms (SNPs) are are believed to be the most common way of acquiring resistance. Mutations in the genes inhA, katG, and rpoB are responsible for the emergence of bacilli resistance to isoniazid and rifampicin, the main drugs used in the treatment of the disease. The gold standard technique for identifying resistant strains is culture, but the result of this test can take up to 8 weeks. Molecular tests like qPCR are faster, but require adequate infrastructure for execution.

Objectives: As an alternative to diagnose tuberculosis in the shortest time possible, and considering the scenario of multidrug resistance, and the beginning of the patient's isolation process, this work aims to optimize a complete point-of-care diagnostic platform, composed of a simplified DNA extraction protocol, a portable qPCR equipment and qPCR reagents optimized to detect M. tuberculosis DNA and the most prevalent mutations in the occurrence of resistant forms, which are the C/T mutations in the inhA gene, and G/ C and G/A mutations in the katG gene.

Methodology: Synthetic gene sequences and genomic DNA extracted from inactivated strains were used for qPCR optimization in the portable instrument. Pre-characterized sputum samples were used to evaluate this optimization. Pre-characterized patient samples stored on FTA cards will be evaluated for the presence of the aforementioned mutations.

Results: Results show that the optimization of the three reactions in the Q3-Plus achieved clinically relevant detection limits when compared to culture and bacilloscopy tests.

Conclusion: These results are promising for the validation of a complete and portable molecular test for detecting antibiotic resistance in M. tuberculosis, suggesting the possibility of early detection and better targeting of patient treatment even in environments with poor infrastructure.

Keywords: point of care diagnosis, antibiotic resistance, tuberculosis