VERIFICATION OF THE NEXT-GENERATION TARGETED SEQUENCEING METHOD USING THE DEEPLEX MYC-TB REAGENT KIT (GENOSCREEN, LILLE, FRANCE) FOR DETERMINING DRUG RESISTANCE OF MICOBACTERIUM TUBERCULOSIS IN SPUTUM SAMPLES

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Abstract

The aim of the study is to verify next-generation targeted sequencing (tNGS) using the Deeplex Myc-TB reagent kit for further implementation into routine practice to accelerate the delivery of drug susceptibility test results, including to an expanded list of anti-TB drugs for effective treatment of tuberculosis.

Material and methods. To verify the new tNGS method using the Deeplex Myc-TB reagent kit, sputum samples were selected that could be further included in targeted sequencing examination. For this purpose, routine phenotypic and genotypic tests of sputum from patients were performed.

tNGS test was conducted using Deeplex Myc-TB kits to detect M. tuberculosis DNA and identify mutations associated with resistance to isoniazid, rifampicin, ethionamide, pyrazinamide, ethambutol, streptomycin, kanamycin, amikacin, capreomycin, levofloxacin, moxifloxacin, linezolid, clofazimine, and bedaquiline. Targeted NGS was performed according to standard operating procedures. Analysis of sequencing data files in FASTQ format was performed using the Deeplex Web Application. Statistical analysis methods were used in the study.

Results. The amount of isolated M. tuberculosis bacterial DNA directly depends on the bacterial load of the sample. The more acid-fast bacteria (AFB) are present in the sample, the more M. tuberculosis bacterial DNA is isolated. The analytical sensitivity of the tNGS method using the Deeplex Myc TB kit depends on the level of bacterial load in native sputum samples. At high load (3+), the sensitivity was 100 % (10/10), at medium load (2+) — 90,0 % (9/10), and at low load (1+) — 70,0 % (7/10). These data indicate the effectiveness of the method for samples with CBC (3+) and CBC (2+), however, with a decrease in the bacterial load, a certain decrease in the sensitivity of the tNGS method using the Deeplex Myc TB kit is noted.

The accuracy, sensitivity and specificity of the tNGS method in detecting resistance of M. tuberculosis to the main anti-tuberculosis drugs were assessed. The test results demonstrate the high efficiency of the tNGS method for determining drug susceptibility to anti-tuberculosis drugs.

The amount of isolated M. tuberculosis DNA for targeted sequencing depends on the bacterial load of the smear: at AFB3+ (high load) the sensitivity was 100%, at AFB2+ (medium) — 90.0%, and at AFB1+ (low) — 70.0%. The results obtained demonstrate the effectiveness of the method for routine diagnostics of samples where the bacterial load is estimated as AFB2+ or AFB3+.

The effectiveness of the new Deeplex Myc-TB test was determined. The minimum indicators compared to fTMS were: 90.0% sensitivity, 91.7% specificity, overall accuracy not lower than 96.9%.

Conclusions. The accuracy and efficiency indicators obtained in the study allow us to recommend tNGS as a method for determining drug resistance M. tuberculosis to 1-st and 2-nd line anti-tuberculosis drugs. The method has been verified for use on sputum samples.

For new and repurposed drugs, the evidence base for mutations remains quite limited, which limits the potential of tNGS as an indepen-

dent diagnostic method. As a result, countries remain dependent on fTMS for these drugs.

For rapid diagnosis and effective treatment in high-burden settings, and guided by new WHO recommendations, the possibility of implementing tNGS into existing diagnostic algorithms should be considered.

Key words: targeted next-generation sequencing, M. tuberculosis, method verification.

Ukr. Pulmonol. J. 2025;33(1):23–30.

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