

## MODERN LABORATORY METHODS FOR DETERMINING TUBERCULOSIS

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**Key words:** tuberculosis (TB), Mycobacterium tuberculosis (MTB), molecular genetic methods, drug resistance, Primary Health Care (PHC).

**Tayanch soʻzlar:** sil (TB), sil mikobakteriyalari (SMB), molekulyar-genetik usullar, dorilarga chidamlilik, birlamchi tibbiy yordam (BTSYO).

**Ключевые слова:** туберкулез (ТБ), микобактерии туберкулеза (МТБ), молекулярно-генетические методы, лекарственная устойчивость, первичная медико-санитарная помощь (ПМСП).

At the present stage, the epidemiological state of tuberculosis remains a concerning problem. The purpose of our research is to study laboratory methods for diagnosing tuberculosis at the present stage and its significance as one of the main factors in improving public health in the structure of infectious diseases in Samarkand region. The article presents data from a literature review and regulatory documents, as well as the implementation of high-tech laboratory tests and accelerated bacteriological methods with the possibility of determining the minimum concentration of mycobacteria in pathological material. The stages of disease diagnosis in patients at various levels of medical and diagnostic institutions in the Samarkand region have been identified. Summarizing our work, it is necessary to emphasize the importance of the ongoing reforms in the healthcare sector in the Republic of Uzbekistan. Timely adequate complex treatment is key to recovery and reduction of disability in tuberculous lesions of various organs and systems of the patient.

## TUBERKULYOZNI ANIQLASHNING ZAMONAVIY LABORATORIYA USULLARI

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Hozirgi bosqichda sil kasalligining epidemiologik holati xavotirli muammo boʻlib qolmoqda. Tadqiqotning maqsadi Samarqand viloyati hududida yuqumli kasalliklar tarkibida zamonaviy bosqichda sil kasalligini tashxislashning laborator usullari va uning aholi salomatligini yaxshilash omili sifatidagi ahamiyatini oʻrganishdan iborat. Maqolada adabiyotlar sharhi va meʼyoriy hujjatlar maʼlumotlari keltirilgan. Patologik materialda mikobakteriyalarning minimal konsentratsiyasini aniqlash imkoniyatiga ega yuqori texnologik laboratoriya testlari va tezlashtirilgan bakteriologik usullarni joriy etish. Samarqand viloyatining turli darajadagi tibbiy-dagnostika muassasalarida bemorda kasallikni tashxislash bosqichlari ajratilgan. Ishlarimizni yakunlar ekanmiz, Oʻzbekiston Respublikasida sogʻliqni saqlash sohasida amalga oshirilayotgan islohotlarning muhimligini taʼkidlash lozim. Oʻz vaqtida oʻtkazilgan adekvat kompleks davolash bemorning turli aʼzolari va tizimlarining sil bilan zararlanishida sogʻayish va nogironlikni kamaytirishning kaliti hisoblanadi.

## СОВРЕМЕННЫЕ ЛАБОРАТОРНЫЕ МЕТОДЫ ОБНАРУЖЕНИЯ ТУБЕРКУЛЁЗА

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На современном этапе эпидемиологическое состояние туберкулёза остаётся тревожной проблемой. Целью нашего исследования было изучение лабораторных методов диагностики туберкулёза на современном этапе и его значение как фактора улучшения общественного здравоохранения в структуре инфекционных заболеваний в регионе Самаркандской области. В статье представлены данные обзора литературы и данные нормативных документов. Внедрение высокотехнологичных лабораторных тестов и ускоренных бактериологических методов с возможностью определения минимальной концентрации микобактерий в патологическом материале. Выделены этапы диагностики заболевания у пациента на различных уровнях медико-диагностических учреждений Самаркандской области. Подводя итоги нашей работы, необходимо подчеркнуть важность проводимых в Республике Узбекистан реформ в области здравоохранения. Своевременное адекватное комплексное лечение является ключом к выздоровлению и снижению инвалидности при туберкулезных поражениях различных органов и систем пациента.

The relevance of the study corresponds to the modern requirements of the fight against tuberculosis (TB), as timely detection, correct diagnosis and treatment, with a guarantee of cure and recovery of the patient (Decree of the President of the Republic of Uzbekistan, from 20.01.2023, № PD-12 on measures for the further development of the service of phthisiatry and pulmonology in 2023 - 2026 years). The coverage of express genetic molecular diagnostics, determining the resistance of MBT to first-line drugs and, in special cases, to first- and second-line anti-tuberculosis drugs, amounts to 120 thousand studies per year.

To improve the quality of life and teach the basics of preventing exacerbations of patients with non-specific lung diseases by organizing “asthma and chronic obstructive pulmonary disease schools” based at multidisciplinary central polyclinics [4-8] organized by the new diagnostics group of the Stop Tuberculosis Partnership, the Foundation for Innovative Diagnostics (FIND), the Global Laboratory Initiative (GLI), the World Health Organization (WHO), NainLifescienceGmbH and non-governmental agencies have developed 11 new diagnostic methods for TB and MDR TB [1-3,5,9].

The modern method of diagnostics of specific infection - polymerase chain reaction, which is based on the determination of pathologic DNA of *Mycobacterium tuberculosis*, has been widely used in the last decade. Modern methodological recommendations provide the latest data on molecular-genetic express methods for detection the resistance to the main drugs isoniazid and rifampicin, which allow to detect multidrug-resistant tuberculosis (GenoTypeMTBDRplus, GenoTypeMTBDRplus 2.0 with MBT and extensively drug resistant tuberculosis (GenoType MTBDR-SI) and differentiate tuberculosis strains from atypical mycobacteriosis (GenoType *Mycobacterium CM*)[4,6-9]. Under the leadership of our government and the Republican Scientific Center of Pulmonology and Tuberculosis, large-scale work is being carried out to combat tuberculosis. Throughout the republic and in particular in Samarkand region according to the order of the Ministry of Health of the Republic of Uzbekistan (№ 383 from 24.10.2014), new innovative high-tech diagnostic methods, successful treatment and prevention schemes of TB are gradually introduced.

**Purpose of the study.** The aim of our study is to investigate the resolving ability of laboratory methods of tuberculosis diagnostics at the present stage, and its importance as one of the factors of population health improvement in the structure of infectious diseases in the region of Samarkand province.

**Materials and methods of research.** We analyzed the existing laboratory methods of primary care diagnostics and new innovative methods of TB diagnostics in Samarkand regional center of phthisiology and pulmonology for the last 10 years. At the stage of primary treatment and diagnostic institutions in the diagnosis of tuberculosis is mainly used bacterioscopic method of examination of pathological material of Cyl-Nielsen staining, which has a relatively low resolution (sensitivity of 25-65% compared to the bacteriological method), but is easy to perform for primary treatment and diagnostic link and not expensive method of research for our contingent of patients [1,4-5]. At the outpatient level, general practitioners and phthisiatrists in primary health care facilities perform the following minimal diagnostic examinations if respiratory tuberculosis is suspected in a patient: timely detection of specific lung disease among the population is carried out according to the specified algorithm.

The Diagnostic algorithm for detection the pulmonary tuberculosis in level III and IV laboratories detail the step-by-step performance of laboratory examination methods: 2-fold sputum examination: portion A - Xpert MTB RIF/ Ultra + CUB microscopy, portion B - seeding + CUB microscopy; review radiography of the chest organs in 2 projections; if indicated, additional and functional methods of examination are carried out: immunologic tests - Mantoux test, Diaskin test. Biochemical blood tests - creatinine, ALT, AST, blood glucose, C-reactive protein; blood tests for HIV; study of external respiratory function (ERF); MSCT of the chest and mediastinal organs; electrocardiographic examination (ECG). At the dispensary level, since 2015, luminescence microscopy (LM) and culture methods have been effectively used according to the diagnostic algorithm: GeneXpert MTB/RIF (Xpertc) automated polymerase chain reaction (PCR) test analysis, 2018 GeneXpert MTBDRplus (HAIN-test), since 2019 VASTES MGIT 960 automated system for bacteriological testing. The GeneXpert® MTB/Rif technology is based on a multiplex amplification system that allows real-time detection of *M. tuberculosis* complex DNA and sensitivity to the anti-tuberculosis drug rifampicin. Patients who come to the dispensary department for examination with suspected pulmonary TB are examined by luminescence microscopy, which has a higher resolution with more accurate results, which is 10% more accurate compared to Cyl-Nielsen staining. However, luminescence microscopy requires special conditions where organic dyes and a binocular microscope with additional illumination are used. All patients are sensitized to antibacterial drugs when applying to the dispensary, for emergency detection of TB pathogen with simultaneous determination of its resistance to rifampicin, a highly sensitive, specific and rapid method, the GeneXpert MTB/RIF and HAIN-test katridge technologies are used. At the stage of inpatient treat-

ment to determine the effectiveness of treatment pathological material of the patient (sputum, bronchial lavage water, fistula secretions and surgical material, various liquids; pleural, cerebrospinal, abdominal, urine) is examined for detection of MBT DNA by PCR methods to all existing ABPs both first and second line, which give the opportunity to obtain a result within no more than 24 hours. Before starting treatment, patients undergo molecular genetic methods of diagnostics of MDR-MBT to rifampicin (the presence of resistance to rifampicin does not exclude the probability of resistance to isoniazid as well), and in case of multiple MDR (MDR) to drugs of reserve series and fluoroquinolones. Taking into account the wide spread of MDR-TB and extensively drug-resistant (XDR-TB) cases, in order to obtain data on the whole spectrum of MBT resistance, one-stage testing of drug sensitivity to PTPs of the first and second line is performed.

VASTES MGIT 960 device with liquid nutrient media is also used to determine the drug sensitivity of MBTs to PTPs of I series (isoniazid, rifampicin, pyrazinamide, streptomycin, ethambutol) and II series (ethionamide, prothionamide, levofloxacin, ofloxacin, amikacin, cycloserine, capreomycin). Seeding of pathologic material has high sensitivity with the ability to detect a lower bacterial load with 10-100 MBT in 1ml. In case of low MBT activity, more information can be obtained by phenotypic determination of the drug susceptibility test (DST).

The bacteriologic method offers more possibilities in the diagnosis of MBT in pathologic material, with a minimum number of pathogens with data to detect the response of an isolate to a particular drug at a minimum concentration, also known as the minimum inhibitory concentration (MIC). The tests are based on measuring the growth of *M. tuberculosis* in liquid (MGIT) or solid (Lowenstein-JensenLY) nutrient media containing a “critical” concentration of drug. The results of HDT are usually available approximately 4 weeks after a positive culture result. For the detection of drug resistance, MGIT and LY on HDT have the same diagnostic value. In the initial stages of laboratory evaluation, LY on MGIT is performed, given the advantages of performing in a short period of time. However, for quality control accuracy and monitoring, LY THR is considered the most informative. In the diagnosis of extrapulmonary tuberculosis, sputum culture is more effective than sputum smear, because of the scanty isolation of MBT. The resolving power of the bacteriologic method is 10-100 mycobacteria in one ml of pathologic material. Diagnostics and differential diagnostics of extrapulmonary tuberculosis with detection of MBT in pleural exudates, in cerebrospinal fluids, in abdominal cavity fluids in surgical material in uncomplicated course of extrapulmonary tuberculosis is improved.

**Results and discussions.** At the present stage, the laboratory of the regional center of our dispensary is equipped with the latest equipment with a high level of sensitivity and efficiency of resistance detection. There are three types of genotypic tests with cartridge system: Xpert MTB/RIF, Hain LPA for detection of MDR-TB (Hain MDRTBplus) and Hain LPA for detection of drug resistance to 2nd-line drugs (Hain MTBDRsl). The time to run the tests is maximized in 24 hours. The test is based on the amplification of DNA of infectious bacteria, the detection of mutations in the *rpoB* gene, with a coverage of up to 95-97%. The sensitivity is up to 98%, and in comparison with the seeding method the sensitivity and specificity are up to 98%. One of the main advantages of molecular genetic methods is high informativeness and rapidity, i.e. in a short period of time it is possible to obtain accurate results with simultaneous determination of sensitivity to all existing anti-tuberculosis drugs of I and II series. Determination of LU on the basis of mutations in associated genes allows timely correction of chemotherapy.

In case of low MBT activity, more information can be obtained by phenotypic method of determining the test for THR. Bacteriological method gives more possibilities in diagnostics of MBT in pathological material, with minimal number of pathogens with data to detect the reaction of isolate to a certain drug in minimal concentration, also known as minimal inhibitory concentra-

1 table.

Main epidemiologic indicators of tuberculosis in Samarkand province:

№	Study Period	Incidence	Morbidity	Mortality
1	2021	49,5	124,28	5,59
2	2022	51,9	10,0	1,1
3	2023	42,55	96,8	1,2
4	2024	41,7	71,7	0,5

tion.

Analyzing the data of epidemiological indicators of Samarkand TB Dispensary for the period from 2021 to 2024, we can see a clear decrease in mortality by 0.5, morbidity by 71.7, morbidity by 41.7 in relation to 100000 population (Table 2). Socio-economic conditions, ecology, migration processes, decline in living standards of the population, increase in the number of unemployed and persons without a fixed place of residence, spread of HIV infection, ineffective work of TB control programs, which leads to the emergence of drug-resistant forms of pathogens, contribute to the deterioration of the tuberculosis situation. The use of accelerated, high-tech diagnostic methods is of great importance for significant improvement of epidemiologic indicators. Application of high technology for TB diagnostics based on PCR has accelerated the process of diagnostics and timely initiation of comprehensive treatment methods. Step-by-step implementation and effective use of the above-mentioned measures, both at the primary stage in the process of examination of the patient in the dispensary to detect the disease and differential diagnosis, and in determining the schemes and control of treatment allowed to reduce the morbidity and mortality from TB and improve the quality of life of the patient.

**Conclusions:** Summarizing the results of our work, it is necessary to emphasize the importance of ongoing reforms in health care in the Republic of Uzbekistan, one of which is the introduction of high-tech laboratory methods of research in medicine. GeneXpert® MTB/Rif technology using the newest system with multiplex amplification allows detection of M.tuberculosis complex DNA and sensitivity to anti-tuberculosis drugs in real time. Culture method is carried out by VASTES MGIT 960 with liquid nutrient media for antibacterial drugs of I series and II series. Accelerated diagnosis and timely adequate comprehensive treatment are the key to recovery and reduction of disability in tuberculosis lesions of various organs and systems of the patient.

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