

**DESCRIPTION OF THE PARAMETERS FOR ASSESSING
MYCOBACTERIUM TUBERCULOSIS USING BACTERIOLOGICAL
METHODS.**

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Keywords: tuberculosis, prevalence, multidrug resistance, extensive drug resistance.

Abstract: Tuberculosis is known to be one of the most widespread infections in the world, affecting humans and animals. Its causative agent, *Mycobacterium tuberculosis* and related pathogens, was discovered by R. Koch and has been proven by numerous researchers. Although the disease caused by this pathogen can be localized in various organs, the pathological process is predominantly localized in the lungs. Differentiating the pathogen among the 74 currently existing species of mycobacteria is extremely difficult, so bacteriological diagnosis of tuberculosis is given considerable attention.

Considering that the pathogenicity and virulence of pathogens vary under the influence of environmental factors, the condition of the host, and, accordingly, certain other biological characteristics, it becomes clear that identification of the causative agent is crucial in the diagnosis of this disease. The presence of an endogenous reactive process in tuberculosis makes bacteriological diagnosis of this disease essential. Taking into account the above, the goal was to conduct a comparative study of the level of detection of the tuberculosis pathogen by microbiological methods and to make a well-founded conclusion, interpreting the results obtained.

**ОПИСАНИЕ ПАРАМЕТРОВ ДЛЯ ОЦЕНКИ НАЛИЧИЯ
MYCOBACTERIUM TUBERCULOSIS С ИСПОЛЬЗОВАНИЕМ
БАКТЕРИОЛОГИЧЕСКИХ МЕТОДОВ.**

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Аннотация: как известно, туберкулёз – одна из самых распространённых инфекций в мире, проявляющаяся инфекционным заболеванием человека и животных. Его возбудитель – микобактерия

туберкулёза и родственные ей возбудители – был открыт Р. Кохом и доказан трудами многих исследователей. Хотя локализация заболевания, вызываемого этим возбудителем, наблюдается в различных органах, патологический процесс преимущественно локализуется в лёгких. Дифференцировать возбудителя среди 74 существующих на сегодняшний день видов микобактерий весьма сложно, поэтому бактериологической диагностике туберкулёза уделяется большое внимание.

Если учесть, что патогенность и вирулентность возбудителей изменяются под влиянием факторов внешней среды, состояния макроорганизма и, соответственно, некоторых других его биологических характеристик, то становится очевидным, что идентификация возбудителя данного заболевания занимает ведущее место в диагностике данного заболевания. Наличие эндогенного реактивного процесса при туберкулезе делает бактериологическую диагностику этого заболевания обязательной.

Учитывая вышеизложенное, была поставлена цель провести сравнительное исследование уровня выявления возбудителя туберкулеза микробиологическими методами и сделать обоснованное заключение, интерпретируя полученные результаты.

Materials and Methods: The object of the study consisted of patients with tuberculosis who were permanent residents of the Bukhara region and received treatment at the Bukhara Regional Center of Phthisiology and Pulmonology. A total of 315 patients were included in the study, the majority of whom were elderly. Among the examined patients, 195 (61.90 ± 2.73%) were men and 120 (38.10 ± 2.73%) were women. Patients aged 50–70 years predominated in the study population, accounting for 67.62 ± 2.64% (n = 213) of all cases.

At present, among the various methods used for the diagnosis of tuberculosis, bacteriological methods are considered one of the most important. Without etiological verification of the disease, the prescription of adequate anti-tuberculosis therapy and the assessment of its effectiveness are significantly limited.

This method enables identification of the causative agent and confirmation of the established clinical diagnosis. Although the bacteriological detection rate of various infectious diseases is relatively low (up to 36.0%) (Nuraliev N.A. et al., 2014; Mukhammedov I.M. et al., 2016), the bacteriological method is regarded as

an essential diagnostic tool. This also applies to tuberculosis, which has not lost its relevance and clinical significance to the present day.

Results and Discussion: The causative agent of tuberculosis is *Mycobacterium tuberculosis*. The results of the study on the level of bacteriological detection of tuberculosis indicate that a positive bacteriological result was observed in $85,08 \pm 2,01\%$ ($n = 268$) of cases, whereas a negative bacteriological result was identified in $14,92 \pm 2,01\%$ ($n = 47$) of cases (Table 1).

Parameters of bacteriological detection of the causative agent of tuberculosis, *Mycobacterium tuberculosis*.

Detection of <i>Mycobacterium tuberculosis</i>	Absolute number	Relative frequency (%)
BK+ (positive bacteriological result)	268	$85,08 \pm 2,01$
BK- (negative bacteriological result)	47	$14,92 \pm 2,01$
Total	315	100

It is noteworthy that BK-positive results were detected 5.70 times more frequently than BK-negative results. This can be attributed, first, to the implementation of modern bacteriological methods for pathogen identification; second, to the resistance of the causative agent to environmental factors; third, to an increase in the proportion of strains resistant to anti-tuberculosis drugs; and fourth, to the development of immunity in patients. This phenomenon is explained by a decrease in the activity of the immune system (immune reactivity of the macroorganism).

If the frequency of negative bacteriological examination results ($n = 14,926$) is analyzed, the failure to detect the causative agent during bacteriological testing can be attributed to several factors. First, non-compliance with the rules for sputum collection by the patient (or healthcare personnel), as well as errors during its collection and transportation to the bacteriological laboratory (medical factor). Second, insufficient processing of sputum for bacterioscopic and bacteriological diagnosis in the laboratory, including improper use of flotation and other methods, resulting in the inability to visualize the pathogen within a given microscopic field or the presence of only a very small number of microorganisms in biological samples during bacteriological culture (bacteriological factor). Third, technological errors in the preparation of the primary culture medium for pathogen isolation—Lowenstein–Jensen medium—incorrect incubation of cultures, and inadequate

maintenance for a sufficient incubation period (identification factor). Fourth, the emergence of strains with specific biological characteristics, including virulence and drug resistance, under the influence of various factors within the human body (strain variability factor). Fifth, an incorrect initial diagnosis of the patient and the absence of diagnostic triggers, leading to false-negative results (false-negative result factor).

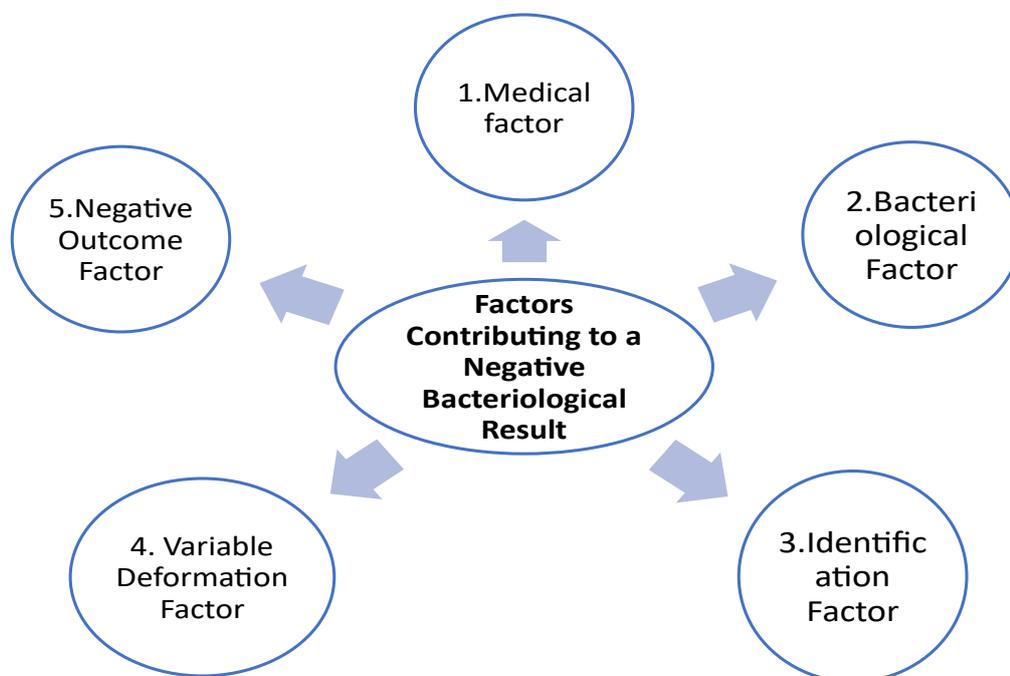


Figure 1. Factors Leading to a Negative Bacteriological Result During the Cultivation of *Mycobacterium tuberculosis*, the Causative Agent of Tuberculosis

It is evident that the bacteriological isolation of *Mycobacterium tuberculosis* is a complex process influenced by numerous objective and subjective factors. Therefore, the high professionalism of all personnel involved in the procedure, the use of complete nutrient media, standardized equipment, and the qualification of the primary diagnosing physician are of great importance.

As a result of the bacteriological studies conducted in this manner, a positive bacteriological result was obtained in 85.08% of cases, while a negative result was observed in 14.92% of cases. This indicates that the bacteriological detection rate of *Mycobacterium tuberculosis* is 5.70 times higher using this method.

For the first time, five factors that can lead to a negative bacteriological result were identified: the medical factor, the bacteriological factor, the identification factor, the strain variability factor, and the false-negative result factor. It was shown that, in the identification and differentiation of the pathogen, along with the high qualifications of the bacteriologist and phthisiatrician, the use

of standardized equipment, proper preparation of culture media, and correct incubation techniques play an essential role.

In addition to bacteriological examination of patient sputum, the Xpert MTB/RIF Ultra method was also employed for pathogen identification. The sensitivity of this method for detecting *Mycobacterium tuberculosis* reaches up to 98.0%, specificity up to 99.0%, and rifampicin (RIF) resistance detection up to 98.0%. Since the advantages and methodology of this novel approach are described in detail in Chapter II, a detailed discussion here was deemed unnecessary. The frequency of positive results obtained by both methods is presented for comparison in Table 2.

Parameters of *Mycobacterium tuberculosis* detection by different methods:

Result	Bacteriological Method		Xpert MTB/RIF	
	Absolute	%	Absolute	%
Positive result	268	85,08± 2,01	269	85,40± 1,99
Negative result	47	14,92± 2,01	46	14,60± 1,99

The results confirm the absence of a practically significant difference in pathogen detection: positive results were $85.08 \pm 2.01\%$ and $85.40 \pm 1.99\%$, and negative results were $14.92 \pm 2.01\%$ and $14.60 \pm 1.99\%$, respectively ($p \leq 0.05$). This indicates that both methods can be effectively used in bacteriological diagnostics.

The Xpert MTB/RIF method is characterized by faster detection compared to the conventional method, while simultaneously indicating strain resistance to rifampicin (RIF). The determination of RIF resistance using this method is presented in Figure 42.

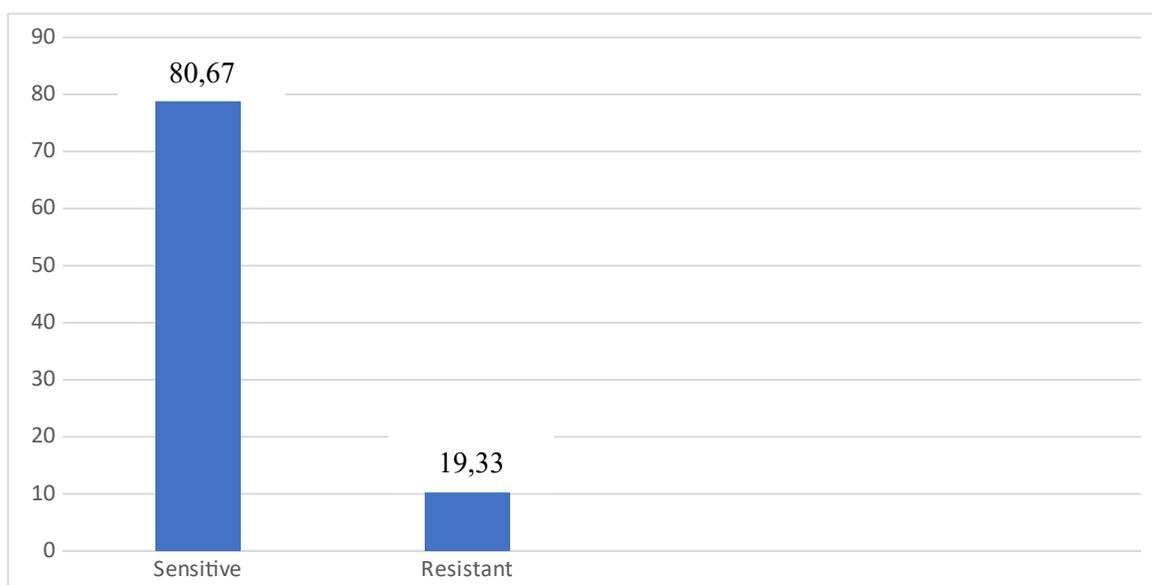


Figure 2. Rifampicin Susceptibility of *Mycobacterium tuberculosis* Strains

As shown in Figure 2, the susceptibility of cultured *Mycobacterium tuberculosis* strains to rifampicin (RIF) was $80.67 \pm 2.41\%$ ($n = 217$), while the detection rate of resistant strains was $19.33 \pm 2.41\%$ ($n = 52$). These values are based on the total number of detected strains ($n = 269$). It is also noteworthy that the proportion of susceptible strains was significantly higher than that of resistant strains, by a factor of 4.17.

Conclusions: Identification of *Mycobacterium tuberculosis* using the Xpert MTB/RIF method resulted in a positive bacteriological outcome in 85.40% of cases and a negative outcome in 14.60%, representing a difference of 5.85 times ($P < 0.001$). This detection rate did not differ significantly from that of the conventional bacteriological method (85.08% positive and 14.92% negative, $P > 0.05$). Among the detected strains ($n = 269$, 85.40%), 80.67% were rifampicin-sensitive, while 19.33% were resistant, with a 4.17-fold higher proportion of sensitive strains.

Consequently, the Xpert MTB/RIF method demonstrated both high sensitivity (98%) and specificity (99%), as well as high bacteriological efficiency. Furthermore, the determination of rifampicin resistance (RIF) enabled rapid and accurate testing, allowing timely and appropriate initiation of anti-tuberculosis therapy.

In recent years, numerous new methods for tuberculosis diagnosis and detection of drug resistance have been recommended. One such method is the HAIN test, which utilizes molecular-genetic techniques to detect resistance of

Mycobacterium tuberculosis to two first-line anti-tuberculosis drugs—isoniazid (H) and rifampicin (R)—directly in patient sputum. The test is performed on a bacteriological smear that has tested positive. Patient sputum is centrifuged and decontaminated using a test strip. The sensitivity of this test is 98.6%, and specificity is 100%, with a positive predictive value of 100% and a negative predictive value of 97.1% (Khamraev A.K., 2012; Chunkaeva D.D., Mansurova D.A., 2017). Another advantage is the very rapid turnaround time: results are available within 5.5 hours, compared to 10–12 days with the MGIT method, or 3–6 weeks using Lowenstein–Jensen medium.

The advantages of using the HAIN test to determine resistance to first-line anti-tuberculosis drugs (H and R) include: (1) rapid detection of drug resistance within 5.5 hours; (2) prevention of the spread of extensive drug resistance within healthcare facilities; (3) early identification of the patient's drug resistance profile; (4) improvement in infection control compliance; and (5) the ability to classify patients based on diagnosis and drug resistance, enabling timely initiation of appropriate treatment.

However, several organizational challenges are associated with this method: (1) laboratory personnel must be appropriately trained and skilled; (2) the equipment used must be standardized, with operation overseen by specially trained technical staff; (3) personnel must monitor processes to prevent contamination and errors; (4) precise quality control must be maintained according to six standards (MGIT); and (5) due to the high cost of this method, its use must be optimized efficiently.

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