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**EXPERIENCE AND EFFICIENCY OF LABORATORY DIAGNOSIS OF TUBERCULOSIS WITH PCR DETECTOR SYSTEM GENEXPERT IN BELGOROD REGION**

**Oleg A. Zemlyansky, Elena B. Tyurina, Andrey A. Bashkirev, Elena V. Kalyuzhnaya, Ludmila O. Zemlyanskaya**

Belgorod State University, 85 Pobedy St., Belgorod, 308000, Russia.

Email: [Zemlynsky@bsu.edu.ru](mailto:Zemlynsky@bsu.edu.ru)

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**Purpose of the Study.** To assess the effectiveness of, and to develop an optimal model for the laboratory diagnosis of tuberculosis using automated PCR detector system GeneXpert.

**Materials and Methods.** The efficiency of a technique to identify the causative agent of tuberculosis in an automated PCR analyzer GeneXpert compared to routine bacteriological methods is analyzed as well as the survey coverage rates of patients and the proportion of TB cases diagnosed in GeneXpert.

**Results.** In the Belgorod region an effective organizational model to identify TB, including MDR, in an automatic PCR analyzer GeneXpert occurred. Coverage of the survey of newly diagnosed patients with pulmonary tuberculosis and relapse exceeded 70%; using GeneXpert revealed more than half of all cases of tuberculosis of smear. Term identify Mycobacterium tuberculosis in GeneXpert averaged 2.3 days from the start of examination of the patient. The efficiency of detection of tuberculosis pathogen is slightly lower than in culture, and is 50.4% of newly diagnosed patients with pulmonary tuberculosis and 69.0% have relapses. The sensitivity and specificity of the pathogen resistance to rifampicin in the sputum of patients with newly diagnosed pulmonary tuberculosis in GeneXpert compared to routine bacteriological methods reach 90-95%.

**Summary and Conclusions.** The introduction of TB services Belgorod region the technique to identify the causative agent of tuberculosis and to determine its susceptibility to rifampicin using automated PCR analyzer the GeneXpert, the development of an optimal model of appliance use allowed in a short time, up to 4 days, with high specificity to provide an etiological diagnosis most epidemiologically dangerous new cases of pulmonary tuberculosis and relapse among patients in the area.

**Keywords:** automatic PCR analyzer the GeneXpert, Mycobacterium tuberculosis, drug resistance, the sensitivity and specificity of the method.

**Introduction.** In the Belgorod region for the past few years is maintained prosperous epidemic situation of tuberculosis. The level of the overall incidence of tuberculosis decreased and in 2013 amounted to 29.3 per 100 thousand population in 2014 - 29.0, in 2015 - 27.1 per 100 thousand population. [1]. Mortality rate from tuberculosis remains the lowest among all the regions of the Russian Federation; in 2015 it was 1.3 per 100 thousand population (Russian Federation - 10.1). [2,3].

However, despite the stability of the epidemiological situation, it remains a high proportion of patients with multidrug-resistant (MDR hereinafter) of the pathogen. So, in 2013 the proportion of MDR among new smear-positive patients was 14.1%, in 2014 - 21.5%, in 2015 - 17.3%; MDR among patients registered on repeated courses of treatment (relapse, failure or interruption of treatment) increased from 48.9% in 2013 to 53.2% in 2014 and amounted to 48.3% in 2015 [4].

The threat of TB MDR in requires the implementation of laboratory methods of rapid detection of *Mycobacterium tuberculosis* and determine its drug resistance.

## **Materials and methods**

In the Belgorod region laboratory diagnosis of all TB cases is carried out centrally on the basis of bacteriological laboratory of the regional TB dispensary, which ensures the accuracy and reduce the time of research, the quality of which is confirmed by the annual participation in FSEQC. The bacteriological laboratory put into practice methods of identifying the causative agent of tuberculosis and to determine its susceptibility to rifampicin using automated PCR analyzer GeneXpert. Given that it is organizational issues largely determine the effectiveness of laboratory diagnostic methods in routine practice, to the laboratory, and TB service area as a whole has been tasked to develop the most optimal usage model GeneXpert analyzer in conjunction with the existing bacteriological methods.

## **Purpose of the study**

To evaluate the effectiveness and to choose the most optimal model for the diagnosis of tuberculosis in an automated PCR analyzer, the GeneXpert, the example of a survey of newly diagnosed patients with pulmonary tuberculosis (hereinafter TB) and relapses registered for treatment in the Belgorod region in 2015.

## **Results and discussion**

In 2015, in the Belgorod region registered for treatment (according to f 07-TB.) 340 newly diagnosed cases of pulmonary TB, of which 201 of smear, and 52 relapsed (41 - MbT). We evaluated: the coverage of the examination of the patient in GeneXpert on diagnostic phase, the efficiency of detection of *Mycobacterium tuberculosis* in

GeneXpert compared with the method of sowing on solid nutrient media Lowenstein-Jensen and Finn-II of, and the proportion of TB cases diagnosed in GeneXpert, the total number of TB MbT.

During the 2015 stage diagnosis towards tuberculosis specialists conducted the material GeneXpert only 199 (58.5%) of new cases of pulmonary TB and 28 (53.8%) recurrences. The reasons of underexamination in some cases were the problems associated with the collection and delivery of the material from remote areas of the region to the laboratory. We survey the organizational model that provides the best possible coverage of patients and rational consumption of reagents was proposed. It lies in the fact that, in addition to aiming phthisiatricians samples were selected on their own laboratory research into GeneXpert aliquot (0.5 ml) of seed rain of diagnostic patients in which at fluorescence microscopy for the first time in a particular patient, to detect any number of acid-fast bacilli ( further AFB). This organizational model enabled promptly examine material from patients who, for whatever reason, are not directed by the attending physician, and the material from paucibacillary patients who had previously negative studies GeneXpert, as Mycobacterium tuberculosis (MBT) detected in them in each sample sputum and very small amounts. With this organizational model, in addition to directional phthisiatricians for 2015 in the Belgorod region surveyed in GeneXpert another 57 new cases of pulmonary TB and 14 recurrences. As a result, coverage of the survey of newly diagnosed patients TBL rose to 75.3%, relapse - to 80.8%.

We have analyzed the effectiveness of the Office in identifying GeneXpert compared with sowing on solid media in newly diagnosed patients with TB and relapses registered for treatment in 2015 (Table 1). The analysis was conducted as part of a series of 3-4 diagnostic sputum samples from a patient prior to treatment.

**Table 1.** The efficiency of detection of the pathogen of tuberculosis in sputum of patients with newly diagnosed and TB backset GeneXpert compared with sowing on solid media

	Patients examined with GeneXpert by swab test	DNA MTB found in GeneXpert	%	MTB found by the swab test	%
Newly diagnosed TB	256	129	50.4	173	67.6
TB backset	42	29	69.0	35	83.3

In 2015, at the stage of diagnosis in GeneXpert and method of planting 256 new cases TB were examined. The MTB found in GeneXpert - in 129 patients, or 50.4%; by culture in 173 patients, or 67.6%.

Out of the 42 examined patients with MTB backset GeneXpert found in 29 patients (69.0%); by culture in 35 (83.3%). Thus, GeneXpert MTB detection efficiency is lower than the swab test. The difference was 17.2% among new cases and 14.3% in relapses.

Both methods showed a higher efficiency among TB relapses, as among these patients, the predominant form of tuberculosis with copious bacterioexcretion.

It should be noted that comparing these two methods is not entirely correct, as they originally are in unequal conditions for the study of multiplicity. Under the current regulatory documentation for diagnostic purposes by culture studied three samples of sputum, in GeneXpert, given the cost of consumables, one or (rarely) two samples. Therefore, in the case of paucibacillary patient or poor-quality sputum it is probable to detect by MTB swab test above.

Like other molecular genetics research methods, GeneXpert gets ahead of the swab test for the production rate results. We analyzed the terms of identifying the causative agent of tuberculosis and its resistance to rifampicin in the GeneXpert, starting from the moment when the patient enters the field of view of a TB.

Of 158 patients (129 new cases and recurrences 29) in which in 2015, it was discovered in a DNA MTB on GeneXpert diagnosis stage, the first day the result was obtained in 57 patients, or 36.1%. In most patients it is stationed in the primary outpatient at a TB in the regional TB dispensary.

In 23 patients (14.6%) MBT DNA detected on the second day from the start of diagnosis. Basically these research materials were directed to rural areas phthisiatricians laboratoies, due to the fact that they require more time for delivery of samples. In 48 patients (30.4%) the result obtained on the third day, in 30 (19.0%) - on the fourth day from the start of diagnosis. Most of these studies carried out aliquots AFB-positive precipitation seed independently selected laboratory. Thus, the timing of detection of the MTB DNA GeneXpert ranged from 1 to 4 days after the beginning of the examination of the patient in a TB. On average, the figure was 2.3 days. Although the short time of the study is very valuable for treating TB specialists, but in practice a number of reasons organizational leads to an increase in diagnostic terms: poor-quality collection of sputum, the problems associated with the delivery of the material in the laboratory, paucibacillary patient.

In 2015, the proportion of newly diagnosed pulmonary TB diagnosed in the GeneXpert, the total number of tuberculosis of smear, was 64.2% (129 of 201 cases); the proportion of relapse - 70.7% (29 of 41 with bacterial relapse). Thus, more than half of epidemiologically dangerous cases of tuberculosis of smear were detected in the first days of diagnosis. This significantly reduced the risk of the spread of tuberculosis, including drug-resistant, among the population of the region. Determine the sensitivity and specificity of the research to identify the causative agent of tuberculosis resistant to rifampicin in GeneXpert compared with the DST method of absolute concentrations on Lowenstein-Jensen medium (Tables 2 and 3). Similar to the previous study, a comparison was carried out in the framework of a series of 3-4 diagnostic sputum samples from a patient prior to treatment.

**Table 2.** Sensitivity and specificity for detection of Mycobacterium tuberculosis resistance to rifampicin in new TB patients in GeneXpert compared with the method of absolute concentrations.

		GeneXpert		
		Resistance to rifampicin	The sensitivity to rifampicin	Total
Absolute concentration method.	Resistance to rifampicin	23	2	25
	The sensitivity to rifampicin	4	100	104
Total		27	102	129
		GeneXpert sensitivity – 92.0%		
		GeneXpert specificity – 96.2%		

In 2015, at the stage of initial diagnosis results of the determination of resistance to rifampicin in GeneXpert and method of absolute concentrations in 129 patients with newly diagnosed pulmonary TB were obtained. Rifampicin resistance is detected by the absolute concentrations in 25 patients, of whom 2 in the survey in GeneXpert rifampicin resistance is not revealed. Sensitivity GeneXpert in these studies was 92.0%. In 4 patients with resistance to rifampicin, identified in the GeneXpert, absolute concentration method of resistance is not found; specificity of research in GeneXpert 96,2%.

**Table 3.** Sensitivity and specificity for detection of Mycobacterium tuberculosis resistance to rifampicin in patients with relapsed TB in GeneXpert in comparison with the method of absolute concentrations.

		GeneXpert		
		Resistance to rifampicin	The sensitivity to rifampicin	Total

Absolute concentration method.	Resistance to rifampicin	12	3	15
	The sensitivity to rifampicin	1	13	14
	Total	13	16	29
		GeneXpert sensitivity – 80.0%		
		GeneXpert specificity – 92.9%		

In 2015, at the stage of diagnosis the results of the determination of resistance to rifampicin in GeneXpert and method of absolute concentrations in 29 patients with relapsed TB were obtained. Rifampicin resistance is detected by the absolute concentrations in 15 patients, of whom 3 in the survey in GeneXpert rifampicin resistance is not revealed; GeneXpert sensitivity in these studies was only 80.0%. In 1 patient with resistance to rifampicin in GeneXpert method of absolute stability is not detected concentrations; GeneXpert specificity of research in comparison with the method of absolute concentrations was 92.9%. The relatively low rate sensitivity in GeneXpert research to identify mutations associated with resistance to rifampicin (80.0%) among the relapse may be due to the fact that patients who have previously performed TB treatment, the possible presence of multiple strains of Mycobacterium tuberculosis.

**Summary and Conclusions**

In the Belgorod region effective organizational model TB detection in an automated analyzer GeneXpert is applied.

In a cohort of 2015 coverage survey of new cases of pulmonary TB was 75.3%, relapse - 80.8%.

The effectiveness of the MTB detection in newly diagnosed patients by GeneXpert was 50.4%, which is 17.2% lower than the swab test. In this recurrence rate was 69.0%, which is 14.3% lower than the swab test.

Term identifying the causative agent of tuberculosis and the mutations associated with resistance to rifampicin in GeneXpert averaged 2.3 days from the start of examination of the patient. More than half of epidemiologically dangerous cases of tuberculosis with bacterial (64.2% of new cases of pulmonary TB and 70.7% recurrence) were identified in the first diagnostic GeneXpert days.

Sensitivity and specificity for detection of Mycobacterium tuberculosis resistance to rifampicin in new patients in TBL GeneXpert compared with the method of absolute concentrations were, respectively, 92.0% and 96.2%; in patients with relapsed TBL - 80.0% and 92.9%.

Thus, implementation of the TB service area method to identify the causative agent of tuberculosis and to determine its susceptibility to rifampicin as a genetic marker of MDR via automatic PCR analyzer the GeneXpert, the

an etiological diagnosis most dangerous epidemic of new cases of pulmonary TB and relapse in the area.

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