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Original Article

A Comparison of Intradermal Test with Recombinant Tuberculosis Allergen (Diaskintest) with Other Immunologic Tests in the Diagnosis of Tuberculosis Infection

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Abstract

Background: The WHO strategy for eradication of tuberculosis (TB) by 2035 (The End TB Strategy) is aimed at an early and precise diagnosis and subsequent effective treatment of TB patients. Currently, there is no gold standard for the diagnosis of latent TB infection. This study evaluated the diagnostic capabilities of a new intradermal test using recombinant TB allergen (Diaskintest) compared with tuberculin skin test (TST) and commercial TB interferon-gamma release assays (IGRAs). **Methods:** A *post-hoc* data analysis that involved examining 860 HIV-negative, bacillus Calmette–Guérin (BCG)-vaccinated persons aged 1–65 years who visited the TB health-care institutions of Saint Petersburg to rule out or confirm an active TB was conducted from 2011 to 2016. **Results:** A high degree of consistency of the Diaskintest results with the enzyme-linked immunospot and QuantiFERON-TB Gold In-Tube test (ELISPOT and QFT) results was observed in the examined pediatric population ($n = 696$), with a Diaskintest cutoff ≥5 mm: the kappa consistency indices were 1.000 and 0.937, for ELISPOT and QFT, respectively. A high sensitivity of Diaskintest, comparable with the IGRA tests, was observed in patients with a confirmed TB diagnosis in all age groups. The sensitivity of Diaskintest in patients of the TB/MTB + group aged 18 years and older was 88.7%; of ELISPOT, 90.6%; of QFT, 87.0%. The conducted analysis has shown a high concordance of results of the commercial TB tests in adult HIV-negative patients ($n = 164$) with a Diaskintest cutoff ≥5 mm: the kappa indices were 0.805 and 0.636 (Diaskintest vs. ELISPOT and QFT, respectively) among BCG-vaccinated people. **Conclusion:** According to the WHO recommendations, replacing the TST by IGRAs is not recommended as a public health intervention in resource-constrained settings because the IGRA tests are more costly and technically complex to conduct than the TST. Diaskintest has comparable complexity to the TST and its performance is close to that of IGRA in a BCG-vaccinated population. Thus, our study demonstrates that replacing the TST by Diaskintest can be recommended as a public health intervention in resource-constrained and universal BCG vaccination settings.

Keywords: Diagnosis of tuberculosis, Diaskintest, immunologic tests, interferon-gamma release assay tests, test with recombinant tuberculosis allergen

Introduction

Tuberculosis (TB) remains one of the most prevalent and dangerous human infections.[1-4] Despite long-term studies and global programs aimed at the management of this disease (DOTS Program, End TB Strategy, and End TB Global Plan for 2006–2015), the infection still has not been defeated.[2,4,5]

In 2013, 9.0 million people developed TB and 1.5 million people died of the disease; in 2014, the disease was detected in 9.6 million people; in 2015, the disease was already detected in 10.4 million people (5.9 million men, 3.5 million women, and 1 million children). In 2015, about 1.8 million people died of TB,

Access this article online

Quick Response Code:

Website: www.ijmyco.org

DOI: 10.4103/ijmy.ijmy_17_18

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How to cite this article: Starshinova A, Zhuravlev V, Dovgaluk I, Panteleev A, Manina V, Zinchenko U, *et al*. A comparison of intradermal test with recombinant tuberculosis allergen (Diaskintest) with other immunologic tests in the diagnosis of tuberculosis infection. Int J Mycobacteriol 2018;7:32-9.

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including 390,000 HIV-positive patients in 2015.[1,2,6] TB is the ninth leading cause of death worldwide and the leading cause involving a single infectious agent, ranking above HIV/AIDS. In 2016, there were an estimated 1.3 million TB deaths among HIV-negative people (down from 1.7 million in 2000) and an additional 374,000 deaths among HIV-positive people. An estimated 10.4 million people fell ill with TB in 2016: 90% were adults, 65% were male, and 10% were people living with HIV. A total of 6.3 million new cases of TB were reported (up from 6.1 million in 2015), equivalent to 61% of the estimated incidence of 10.4 million. There were 476,774 reported cases of HIV-positive TB (46% of the estimated incidence).

According to the WHO, one-third of the world's population is infected with *Mycobacterium tuberculosis* (MTB).

The WHO strategy for eradication of TB by 2035 (The End TB Strategy) is aimed at an early and precise diagnosis and subsequent effective treatment of TB patients.[2] Currently, there is no gold standard for the diagnosis of latent TB infection (LTBI) and early detection of active TB.

For a long time, the Mantoux test was the only immunologic method of early diagnosis of TB infection.[7-9]

The tuberculin skin test (TST, Mantoux test) has been known since the late 19th century and is based on a delayed hypersensitivity reaction to specific proteins of the pathogen. Tuberculin was approved by the WHO in 1958 and contains >200 antigens common in the TB and non-TB mycobacteria (NTM).[7] As a result, a positive immune response to tuberculin administration is observed in persons sensitized with NTM or immunized with bacillus Calmette–Guérin (BCG). It is extremely difficult to perform TB diagnosis based on the Mantoux test in countries with BCG immunization, which leads to low diagnostic specificity of the test.[10]

Tests based on interferon-γ release induction (interferon gamma release assay [IGRA]), based on the stimulation of a cellular immune response by immunodominant antigens ESAT-6 and CFP10 specific to MTB, represent a diagnostic alternative to the tuberculin test; this solves the problem of sensitization by BCG immunization and NTM infection, which is a factor when using TST.[11]

Two IGRA test options have been introduced recently in international practice: QuantiFERON-TB Gold In-Tube test (QFT, Qiagen, Hilden, Germany), where whole blood is used, and T-SPOT.TB test (enzyme-linked immunospot [ELISPOT], Oxford Immunotec, UK, with the use of purified peripheral blood mononuclear cells). According to various studies, their informational value in TB diagnosis is quite high: 78%–93%.[12-14] The emergence of IGRA tests has allowed to better identify hidden LTBI.[13,15]

At the same time, the high cost of these tests prevents WHO from recommending them in low-income countries.[2] A need for a specially equipped laboratory, trained personnel, and intravenous manipulations is a substantial drawback of the IGRA tests. These limitations make it impossible to use the IGRA tests for large-scale screening studies, particularly among children (obtaining of venous blood), in contrast to the TST.

Recombinant TB allergen (Diaskintest) manufactured by GENERIUM JSC, Russia, is a recombinant fusion protein CFP10-ESAT6 produced by *Escherichia coli* BL21(DE3)/pCFP-ESAT.[16] Diaskintest, similar to the TST, is performed *in vivo*. It is based on a delayed hypersensitivity reaction after intradermal administration of specific proteins.[17-19] In contrast to the Mantoux test, the results of Diaskintest are not affected by BCG immunization.[20] According to a wide range of studies, Diaskintest shows high sensitivity and specificity in TB diagnosis.[7,17,18]

The recombinant TB allergen test was registered in the Russian Federation in 2008 based on the results of preclinical studies and clinical trials and was legally approved for complex TB diagnosis in children and adolescents from 2009.[21-23]

We have conducted a study aimed at a comparison of the sensitivity of the intradermal test using recombinant TB allergen (Diaskintest) with other immunologic tests in the diagnosis of active TB infection and LTBI. We have also compared the concordance of the results of the existing immunologic TB tests.

Methods

Study design and participants

A *post-hoc* data analysis was performed at two TB health-care institutions in Saint Petersburg (Russia). The study was approved by the Independent Ethics Committee of the Federal State Research Institute of Phthisiopulmonology of the Ministry of Health of the Russian Federation (Approval No. 16 on April 4, 2014). This study is a retrospective cohort study with an evaluation of the results from all persons who visited the health-care institutions to rule out or confirm a TB diagnosis between January 1, 2011, and December 30, 2016. A medical history, baseline characteristics (i.e., age, sex, BCG immunization, comorbidity, use of immunosuppressants, and a history of TB), and results of bacteriological, radiological, and immunological examinations were evaluated at participants included in the study. Individuals aged 1–65 years, who had undergone BCG immunization, were included in the study. Confirmation of vaccination: Medical files (children), evidence of BCG scar (children and adults).

The study exclusion criteria were as follows: age <1 year and >65 years; a diagnosis of current active TB disease; anti-TB drugs therapy ≥1 month; pulmonary mycobacteriosis; a severe and/or decompensated comorbidity; HIV-positive status; pregnancy and lactation in women; absence of BCG immunization. Individuals with secondary immunodeficiency disorders, such as diabetes mellitus, organ transplantation, and malignancies, and persons receiving corticosteroid treatment were also excluded as well. Thus, 860 individuals

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were selected, with subsequent age stratification: children and adolescents aged 1–18 years and adults aged 18–65 years.

The examination of all individuals, who contacted specialists at the anti-TB institution, was conducted in accordance with the current normative legal documents. First, the clinical symptoms were evaluated. Then, the blood samples for QFT and ELISPOT tests were collected, followed by the Mantoux test with 2 TU (MT/TST) and a recombinant TB allergen test (Diaskintest). Due to the limited capabilities, interferon-γ release tests (IGRA tests) were only performed in some individuals. According to the current routine practice, a radial examination of thoracic organs was performed to all individuals: a chest X-ray (Multix PRO, Germany) and/or a computed tomography of the lungs and organs of the mediastinum (Somatom AS, Germany). A respiratory material examination for MTB was performed with the use of microbiological and bacteriological methods (sputum smear, sputum culture on solid media [Lowenstein–Jensen, Finn 2] and liquid media [BACTEC MGIT 960 analyzer (Becton Dickinson Microbiology System, Sparks, MD)] as well as MTB DNA detection by real-time polymerase chain reaction [RT-PCR, AmpliTub-RV, Russia]).

The general characteristics of the individuals are presented in Table 1.

There were 696 individuals aged <18 years. Mean age was 8.1 ± 3.5 years. Three hundred and forty-five individuals (49.6%) were female, and 351 individuals (50.4%) were male. One hundred and sixty-four individuals aged 18–65 years were also included in the study. The mean age of individuals was 37.0 ± 13.05 years. One hundred and six adult individuals (64.6%) were female, and 58 adult individuals (35.4%) were male. The percentage of patients with active TB confirmed bacteriologically was 2.1% (n = 15) among individuals <18 years and 32.3% (n = 53) among adults aged 18 years and older.

In children (15/15, 100%), the diagnosis of TB is confirmed with molecular genetic methods (RT-PCR). In adults, in 33 patients, MTB was detected with sputum microscopy and in 44 people – with inoculation of liquid/dense media. Among others, a positive sputum smear and positive cultures were concurrently detected in 29 patients. Some patients were tested with molecular genetic methods: a positive RT-PCR result was obtained in 31 people.

The persons were assigned to comparison groups according to the examination results and diagnosis of active TB [Figure 1].

All individuals were stratified by age as children and adolescents aged 1–18 years and adults aged 18–65 years. Groups of patients with confirmed pulmonary TB and individuals without diagnosed active TB were defined based on the examination results. Diagnosing TB is known to be complicated in children and adolescents. In our study, this age group included patients with MTB detected in sputum and patients with TB diagnosed based on the conclusion of a medical board after comprehensive clinical and radiological examinations only (without MTB detection).

Thus, this study included an analysis of results obtained in three subgroups of children and adolescents aged <18 years: Group 1 (n = 15), children and adolescents with active TB, confirmed bacteriologically (TB, MTB+), Group 2 (n = 245), children and adolescents with a TB diagnosis (TB, MTB+) confirmed by clinical and radiological findings, Group 3 (n = 436), children and adolescents in whom no active TB infection was identified by a TB specialist ("without active TB"). Adults aged 18–65 years were divided into two subgroups: patients with active TB, confirmed bacteriologically (TB, MTB+, n = 53) and persons

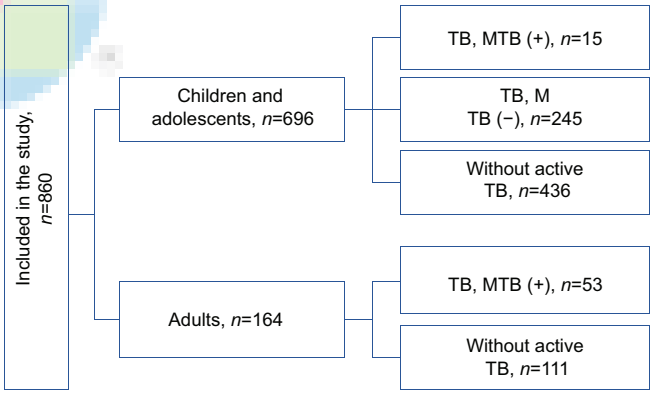

Figure 1: The study design

Table 1: The general characteristics of the population

	Children and adolescents (n=696), n (%)	Adults (n=164), n (%)
Sex		
Female	345/696 (49.6)	106/164 (64.6)
Male	351/696 (50.4)	58/164 (35.4)
Age (95% CI)	8.1±3.5 (7.8-8.4)	37.0±13.05 (34.9-39.1)
BCG immunization	100.0	100.0
Specific changes in X-ray diagnosis, including	260/696 (37.4)	53/164 (32.3)
Pulmonary focal and infiltrates	17/260 (6.5)	53/53 (100)
Intrathoracic lymphadenopathy	257/260 (98.8)	3/53 (5.7)
Bacterial excretion	15/696 (2.1)	53/164 (32.3)

BCG: Bacillus Calmette-Guérin, CI: Confidence interval

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who were not diagnosed with active TB after a comprehensive examination at a TB health-care institution ("without active TB," $n = 111$).

According to study design and to obtain comparison data, immunologic tests (sampling blood for IGRA-tests) were done as the first step; afterward, we conducted drug-susceptibility testing (DST). Results of TST were taken from medical history of the patients.

Study methods

Intradermal tests

Performing a Diaskintest is similar to the TST. The injections were performed intradermally; the results were recorded after 72 h by measuring the papule diameter at the injection site.

According to the guidelines, in the presence of a papule of any size, the Diaskintest results were considered positive. The presence of hyperemia in the absence of a papule was considered as an inconclusive test. A cutoff of ≥5 mm was established in this study for an objective assessment of the presence of a papule.

According to the Russian legislation, TST was performed with the use of Linnikova-purified protein derivative tuberculin with two tuberculin units (Russia, Pharmstandard JSC). The TST results were evaluated as follows: positive – a papule of 5 mm and more, inconclusive – a papule up to 4 mm or hyperemia of any size. The absence of a papule and hyperemia in both tests was a negative parameter.

Immunologic laboratory tests

The ELISPOT test was performed according to the guidelines of the manufacturer (Oxford Immunotec, UK). Purified peripheral blood lymphocytes were incubated with the test antigens using the GIBCO AIM-V™ culture medium (Invitrogen, Paisley, UK). The number of spots in each cell (representing cells producing IFN-γ) was assessed visually using a magnifying glass, by two independent observers who were unaware of the QFT results. The results were interpreted according to the criteria established by the manufacturer for the use of the test outside the United States. A positive result was defined as ≥6 spots in an ESAT-6 cell or a CFP-10 cell after subtraction of the number of spots observed in the negative control cell where the negative control had 0–5 spots. If the negative control had ≥6 spots, the ESAT-6 or CFP-10 panel had to contain at least twice as many spots compared with the negative panel for the result to be considered positive. The result was considered as inconclusive if the negative control cell contained >10 spots or the mitogen control contained <20 spots (with <6 spots in the ESAT-6 and CFP-10 cells).

A QFT was also performed according to the guidelines of the manufacturer. Venous blood was collected from each subject into each of three special evacuated and heparinized containers calibrated for drawing of 1 ml of blood. The kit included a TB-antigen-coated tube, an NIL tube (negative control), and mitogenic (phytohemagglutinin) tube (positive control). According to the recommendations, the cutoff value for the positive test was IFN-γ >0.35 IU/ml for the TB antigen minus NIL. A negative result was recorded if this response was <0.35 IU/ml, and the mitogen control minus NIL was ≥0.5 IU/ml. If IFN-γ was below the respective cutoff values both for the TB Antigen-NIL and mitogen-NIL, the result was considered inconclusive. The maximum IFN-γ level, precisely determined using an EIA with QFT, was 10 IU/ml, so the values exceeding it were reported as 10 IU/ml.

The results of borderline IGRA test were considered as negative due to an uncertain probability of TB infection.[24]

Statistical analysis

The data analysis was performed with the use of the Stata 14 software.[25] Descriptive statistics methods were used to characterize the subjects included in the study. The following was evaluated for the quantitative parameters: arithmetic mean (mean); standard deviation (SD); 95% confidence interval for the mean. The absolute number as n/N as well as the percentage (%) was analyzed for the qualitative variables. A Cochran's Q-test was used for a comparison of the sensitivity of different tests in each group. A pairwise comparison with the results of Diaskintest with a cutoff ≥5 mm was performed for each test. For the analysis, negative, inconclusive, and uninterpreted results were combined into one group. The kappa consistency index, which considers accidental correspondence of the results, was calculated for each pair of tests. A comparison of the frequency of a parameter in unrelated subgroups of patients was performed using Fisher's exact test. The differences between compared subgroups were considered statistically significant at $P < 0.05$.

Study results

Analysis of immunologic test parameters

A statistically significant difference between the results of the four tests was obtained for children and adolescents in the group without active TB ($P < 0.0001$). The same analysis was not performed in subgroups of patients with bacterial excretion or without it due to insufficient data on the patients for whom the results of all four tests were available [Table 2].

As seen in Table 2, a statistically significant difference between the groups with and without active TB was observed using the IGRA tests and Diaskintest. The TST was positive in all patients (100%).

An analysis of concordance of the Diaskintest results in children <18 years and the *in vitro* tests (ELISPOT and QFT) was then performed [Table 3].

The concordance between the Diaskintest and Mantoux test was significantly lower (0.299) due to its lack of the results of skin tests in the group of persons without active TB [Table 4].

According to the analysis, a concordance of the Diaskintest results with the ELISPOT and QFT results was observed in most cases in the examined pediatric population ($n = 696$) with

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Table 2: The results of various diagnostic tests in children under 18 years of age

Group	Test result	ELISPOT, *n* (%)	QFT, *n* (%)	Diaskintest, *n* (%)	TST, *n* (%)	*P* (Q-test)
TB/MTB+	Positive	2/2 (100.0)	12/12 (100.0)	15/15 (100.0)	15/15 (100.0)	-
	Negative	0/2 (0.0)	0/12 (0.0)	0/15 (0.0)	0/15 (0.0)	
	Inconclusive	0/2 (0.0)	0/12 (0.0)	0/15 (0.0)	0/15 (0.0)	
	Not interpreted	0/2 (0.0)	0/12 (0.0)	0/15 (0.0)	0/15 (0.0)	
TB/MTB-	Positive	6/6 (100.0)	118/122 (96.7)	245/245 (100.0)	245/245 (100.0)	-
	95 CI	-	93.5-99.9	-	-	
	Negative	0/6 (0.0)	4/122 (3.3)	0/245 (0.0)	0/245 (0.0)	
	95 CI	-	0.05-6.5	-	-	
	Inconclusive	0/6 (0.0)	0/122 (0.0)	0/245 (0.0)	0/245 (0.0)	
	Not interpreted	0/6 (0.0)	0/122 (0.0)	0/245 (0.0)	0/245 (0.0)	
Without active TB	Positive	24/228 (10.5)	71/178 (39.9)	127/434 (29.3)	436/436 (100.0)	<0.0001
	95 CI	6.5-14.6	32.5-47.2	24.9-33.6	-	
	Negative	204/228 (86.5)	107/178 (60.1)	307/434 (70.7)	0/436 (0.0)	
	Inconclusive	0/228 (0.0)	0/178 (0.0)	0/434 (0.0)	0/436 (0.0)	
	not Interpreted	0/228 (0.0)	0/178 (0.0)	0/434 (0.0)	0/436 (0.0)	
P (Fisher)		<0.001	<0.001	<0.001	-	

CI: Confidence interval, TB: Tuberculosis, MTB: *Mycobacterium tuberculosis*, TST: Tuberculin skin test, QFT: QuantiFERON-TB Gold In-Tube test, ELISPOT: Enzyme-linked immunospot assay

a Diaskintest cutoff ≥5 mm: The kappa consistency indices were 1.000 and 0.937, respectively. The Diaskintest and TST results were at the level of accidental correspondence; however, the concordance of positive values in the group of patients with a confirmed diagnosis was 98.8%–100.0%.

The results of immunologic tests in HIV-negative persons aged 18 years and older

The results of immunologic tests in this group of patients are presented in Table 5.

As seen in Table 5, an analysis using Cochran's Q test has demonstrated the absence of statistically significant differences between the sensitivity parameters of various diagnostic tests in patients with microbiologically confirmed TB ($P = 0.903$). Significant differences between the results of the four tests were only observed in the group of patients without active TB ($P < 0.0001$).

The analysis has shown a generally high concordance of the test results in adult HIV-negative patients: the consistency (kappa) of the Diaskintest results (Diaskintest cutoff ≥5 mm) with the ELISPOT and QFT test results were 0.805 and 0.636, respectively.

Results and Discussion

According to the WHO strategy for eradication of TB by 2035,[1] the level of active TB should be kept under control, but the main focus should be on decreasing the level of LTBI. According to numerous studies, the IGRA tests are highly valuable in the diagnosis of both active and latent TB; however, they are expensive, complicated, require taking venous blood samples and trained laboratory staff, and prevent them from being recommended as screening tests in many countries. The existing TST has low diagnostic value in countries with a high level of BCG immunization, including Russia. Introduction of novel cost-effective tests with high sensitivity is required for LTBI diagnosis. This study evaluated the diagnostic capabilities of a new intradermal test using recombinant TB allergen (Diaskintest).

Considering the features of the pathogenesis of the specific process, a *post-hoc* analysis of the immunologic test results obtained in two cohorts of patients (in children and adolescents and in adults) was performed in countries with BCG vaccination.

A sensitivity of Diaskintest comparable with the IGRA tests was observed in patients with a confirmed TB diagnosis in all age groups.

The kappa consistency indices for the Diaskintest results (Diaskintest cutoff ≥5 mm) versus the ELISPOT and QFT results in the examined pediatric population ($n = 696$) were 1.000 and 0.937, respectively. Diaskintest has demonstrated 100% sensitivity at the level of the existing immunologic tests among children and adolescents with diagnosed TB. An evaluation of the concordance of Diaskintest with IGRA confirms that the new skin test can be used for diagnosing TB. This is confirmed by the high level of concordance of the positive interferon release and Diaskintest test results in patients in whom a diagnosis of the specific process has been ruled out, while the concordance with the TST is low. The differences between the Diaskintest and TST are most probably caused by the existing BCG immunization, to which TST is sensitive. A high consistency of the Diaskintest and IGRA test results in children and adolescents has been observed by other researchers as well.[26]

In this study, the sensitivity of Diaskintest in patients of the TB/MTB+ group aged 18 years and older was 88.7%; of ELISPOT, 90.6%; of QFT, 87.0%. According to some authors, the sensitivity of QFT does not exceed 89.0%.[27]

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Table 3: A comparison of the Diaskin test results with the results of other diagnostic tests in patients aged below 18 years (cutoff ≥5 mm)

Diaskintest	ELISPOT				QFT				Mantoux test			
	Negative	Positive	Agreement (%)	κ	Negative	Positive	Agreement (%)	κ	Negative	Positive	Agreement (%)	κ
Total population												
Negative	204/235 (86.8)	0/235 (0.0)	100.0	-	106/311 (34.1)	5/311 (1.5)	97.1	0.937	0/694 (0.0)	314/694 (45.2)	54.8	0.000
Positive	0/235 (0.0)	31/235 (13.2)			4/311 (1.3)	196/311 (63.0)			0/694 (0.0)	380/694 (54.8)		
TB/MBT+												
Negative	0/2 (0.0)	0/2 (0.0)	100.0	-	0/12 (0.0)	0/12 (0.0)	100.0	-	0/15 (0.0)	0/15 (0.0)	100.0	-
Positive	0/2 (0.0)	2/2 (100.0)			0/12 (0.0)	12/12 (100.0)			0/15 (0.0)	15/15 (100.0)		
TB/MBT-												
Negative	0/6 (0.0)	0/6 (0.0)	100.0	-	1/122 (0.8)	1/122 (0.8)	96.7	0.318	0/245 (0.0)	3/245 (1.2)	98.8	-
Positive	0/6 (0.0)	6/6 (100.0)			3/122 (2.5)	117/122 (95.9)			0/245 (0.0)	242/245 (98.8)		
Without active TB												
Negative	204/227 (89.9)	0/227 (0.0)	100.0	-	105/177 (59.3)	4/177 (2.3)	97.2	0.941	0/434 (0.0)	311/434 (71.7)	28.3	0.000
Positive	0/227 (0.0)	23/227 (10.1)			1/177 (0.6)	67/177 (37.9)			0/434 (0.0)	123/434 (28.3)		

TB: Tuberculosis, MTB: *Mycobacterium tuberculosis*, QFT: QuantiFERON-TB Gold In-Tube test, ELISPOT: Enzyme-linked immunospot assay

Table 4: A comparison of the Diaskintest results with the results of other diagnostic tests in patients aged below 18 years (cutoff ≥5 mm)

Diaskintest	ELISPOT				QFT				Mantoux test			
	Negative (%)	Positive (%)	Agreement (%)	κ	Negative (%)	Positive (%)	Agreement (%)	κ	Negative (%)	Positive (%)	Agreement (%)	κ
Total population												
Negative	204/235 (86.8)	0/235 (0.0)	100.0	-	106/311 (34.1)	5/311 (1.5)	97.1	0.937	0/694 (0.0)	314/694 (45.2)	54.8	0.000
Positive	0/235 (0.0)	31/235 (13.2)			4/311 (1.3)	196/311 (63.0)			0/694 (0.0)	380/694 (54.8)		
TB/MBT+												
Negative	0/2 (0.0)	0/2 (0.0)	100.0	-	0/12 (0.0)	0/12 (0.0)	100.0	-	0/15 (0.0)	0/15 (0.0)	100.0	-
Positive	0/2 (0.0)	2/2 (100.0)			0/12 (0.0)	12/12 (100.0)			0/15 (0.0)	15/15 (100.0)		
TB/MBT-												
Negative	0/6 (0.0)	0/6 (0.0)	100.0	-	1/122 (0.8)	1/122 (0.8)	96.7	0.318	0/245 (0.0)	3/245 (1.2)	98.8	-
Positive	0/6 (0.0)	6/6 (100.0)			3/122 (2.5)	117/122 (95.9)			0/245 (0.0)	242/245 (98.8)		
Without active TB												
Negative	204/227 (89.9)	0/227 (0.0)	100.0	-	105/177 (59.3)	4/177 (2.3)	97.2	0.941	0/434 (0.0)	311/434 (71.7)	28.3	0.000
Positive	0/227 (0.0)	23/227 (10.1)			1/177 (0.6)	67/177 (37.9)			0/434 (0.0)	123/434 (28.3)		

TB: Tuberculosis, MTB: *Mycobacterium tuberculosis*, QFT: QuantiFERON-TB Gold In-Tube test, ELISPOT: Enzyme-linked immunospot assay

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Table 5: The results of various diagnostic tests in the adult subjects aged 18 and over

Group	Test result	ELISPOT, *n* (%)	QFT, *n* (%)	Diaskintest, *n* (%)	Mantoux test, *n* (%)	*P* (Q-test)
TB/MTB+	Positive	48/53 (90.6)	40/46 (87.0)	47/53 (88.7)	45/53 (84.9)	0.903
	95% CI	82.5-98.6	77.0-96.9	80.0-97.4	75.1-94.7	
	Negative	3/53 (5.7)	6/46 (13.0)	6/53 (11.3)	6/53 (11.3)	
	Inconclusive	2/53 (3.8)	0/46 (0.0)	0/53 (0.0)	2/53 (3.8)	
	Not interpreted	0/53 (0.0)	0/46 (0.0)	0/53 (0.0)	0/53 (0.0)	
Without active TB	Positive	6/111 (5.4)	6/62 (9.7)	13/111 (11.7)	66/111 (59.5)	<0.0001
	95% CI	1.1-9.7	2.2-17.2	5.6-17.8	50.1-68.8	
	Negative	98/111 (88.3)	55/62 (88.7)	98/111 (88.3)	44/111 (39.6)	
	Inconclusive	7/111 (6.3)	1/62 (1.6)	0/111 (0.0)	1/111 (0.9)	
	Not interpreted	0/111 (0.0)	0/62 (0.0)	0/111 (0.0)	0/111 (0.0)	
P (Fisher)		<0.001	<0.001	<0.001	<0.001	

CI: Confidence interval, TB: Tuberculosis, MTB: *Mycobacterium tuberculosis*, QFT: QuantiFERON-TB Gold In-Tube test, ELISPOT: Enzyme-linked immunospot assay

Because a diagnosis of LTBI is the endpoint of the study, an evaluation of the consistency of the laboratory tests and Diaskintest by the kappa criterion was performed among patients in whom the diagnosis of active TB had been ruled out. The analysis has demonstrated high consistency of the results of the commercial TB tests in adult patients (Diaskintest cutoff ≥5 mm): The kappa index was 0.805 and 0.636 (Diaskintest vs. ELISPOT and QFT, respectively).

Our data suggest that Diaskintest has sensitivity comparable with that of IGRA and a high percentage of concordance of the test results both in the group with active TB and with regard to the detection of an LTBI. The results obtained confirm that Diaskintest can enable a more substantiated approach to follow-up examinations and treatment of persons as compared with TST among BCG-vaccinated people.

Currently, according to the WHO recommendations, replacing the TST by IGRAs is not recommended as a public health intervention in resource-constrained settings, because the IGRA tests are more costly and technically complex to conduct than the TST. Diaskintest has comparable complexity to the TST, and its performance is close to that of IGRA in a BCG-vaccinated population.

Thus, our study demonstrates that replacing the TST by Diaskintest can be recommended as a public health intervention in resource-constrained and universal BCG-vaccination settings.

It is important to consider favorable cost-effectiveness for DST (if compared to IGRA-tests) while its similar sensitivity with IGRA. Implementation of DST can help improve early diagnosis in the countries with high burden of TB with BCG vaccination.

Financial support and sponsorship

Financial support is providing by Ministry of Health Russian Federation

Conflicts of interest

There are no conflicts of interest.

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