STUDY PROTOCOL

This document contains confidential information.

ASSESSMENT OF THE SPECIFICITY OF THE INTRADERMAL TEST WITH TUBERCULOSIS RECOMBINANT ALLERGEN IN BCG-VACCINATED HEALTHY SUBJECTS

A prospective, multicenter, open-label, cohort study of healthy adults (18 to 30 years of age) (asymptomatic and not at risk for tuberculosis)

Protocol No.	102/1
Name of the investigational medicinal product:	Recombinant tuberculosis allergen (Diaskintest)
Organization responsible for the study:	National Medical Research Center of Tuberculosis, Pulmonology, and Infectious Diseases, a federal state-financed institution of the Ministry of Health of the Russian Federation
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Initial protocol date:	November 22, 2021

1. INVESTIGATOR SIGNATURE PAGE

INVESTIGATOR:

I consent to:

- Implement and conduct this study properly and in strict compliance with the protocol, GCP requirements, all applicable laws, and regulatory requirements.
- Maintain the confidentiality of all information about the study and, when this information is submitted to a local ethics committee or other agencies, it will be filed as confidential.
- Ensure that all persons involved in the study are adequately informed of the protocol, study intervention(s), their responsibilities, and roles in the study.

I have fully read the protocol and agree with all its aspects.

Investigator's first and last name	Signature	Date in block letters

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3. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

RTA	Recombinant tuberculosis allergen
BCG	Bacillus Calmette-Guérin
WHO	World Health Organization
CI	Confidence interval
CRF	Case report form
IFN-γ	Interferon-gamma
LEC	Local Ethics Committee
First Volunteer In	First visit of the first Volunteer
RTA test	Test with recombinant tuberculosis allergen
AE	Adverse event
AR	Adverse reaction
Last Volunteer Out	Last visit of the last Volunteer
SUSAR	Suspected unexpected serious adverse (drug) reaction
Suspected Adverse Reaction Reporting Form	A form of the Council of International Organizations of Medical Sciences
Specificity	Probability to get a negative test result in a subject without the disease
Sensitivity	Probability to get a positive test result in a subject with the disease
The NMRC TPID	National Medical Research Center of Tuberculosis, Pulmonology, and Infectious Diseases, a federal state-financed institution of the Ministry of Health of the Russian Federation
CFP-10	Recombinant culture filtrate protein 10 kDa
ESAT-6	Recombinant early secreted antigenic target 6 kDa
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
ICH-GCP	The International Conference on Harmonisation of Good Clinical Practice
MTb	*Mycobacterium tuberculosis*
T-SPOT®.TB	Diagnostic assay with a reagent kit for in vitro diagnosis of tuberculosis infection T-SPOT.TB, Oxford Immunotec Ltd., United Kingdom

4. SIGNATURE PAGE

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6. INTRODUCTION

Tuberculosis remains the most common infection, affecting more than 10 million people annually and causing an estimated 1.2 million deaths [WHO, Global Tuberculosis report 2020]. For a century, tuberculin has been used worldwide to diagnose tuberculosis and detect latent tuberculosis infection [von Pirquet C., 1907, Mantoux M.,1912]. The major disadvantage of the tuberculin test is a high rate of false positive reactions due to cross-reactivity with PPD antigens that are present in many mycobacteria and strains of Bacillus Calmette-Guérin (BCG). In a meta-analysis of 14 studies from other countries, the pooled sensitivity of the tuberculin test was 71% [1]. In a large review of foreign literature and meta-analysis, Rangaka et al. (2012) indicate that a positive tuberculin reaction has a low prognostic value in assessing the probability of the disease [2].

The limited specificity of the tuberculin skin test is particularly relevant in large-scale BCG vaccination programs and in the screening a large population of tuberculosis-infected patients, i.e. in most countries with high tuberculosis rates. The discovery of Mycobacterium tuberculosis-specific antigens that are absent in Mycobacterium bovis BCG and most mycobacteria resulted in the development of *in vitro* tests measuring gamma interferon (INF-γ) production (Interferon-Gamma Release Assays or IGRA) in response to stimulation with these antigens. The genome of Mycobacterium bovis BCG and most non-tuberculous mycobacteria has no region of difference (RDI) containing genes encoding these proteins. These tests showed high sensitivity and almost absolute specificity.

In 2009, the diagnostic product recombinant tuberculosis allergen (RTA), a recombinant protein CFP10-ESAÒ6 produced by Escherichia coli BL21(DE3)/pCFP-ESAT, was granted marketing authorization in Russia. The Russian skin test with RTA is analogous to the IGRA-based tests. No special laboratory equipment is required to perform the skin test. The RTA test showed high sensitivity (over 95%) in the mass screening of patients in Moscow in 2012–2014 [3,4], as well as in Novosibirsk Oblast [5], Ryazan Oblast [6], and St. Petersburg [7].

The following normative documentation regulates the use of the RTA test in Russia: Order of the Ministry of Health of the Russian Federation No. 855 of October 29, 2009; Order of the Ministry of Health of the Russian Federation No. 951 of December 29, 2014; Order of the Ministry of Health of the Russian Federation No. 124н of March 21, 2017; the clinical guidelines “Latent tuberculosis infection in children,” “Tuberculosis in children” (Moscow, 2020) [10], and the clinical guidelines “Tuberculosis in adults” (Moscow, 2020) [11].

RTA has been used in the Russian Federation and CIS since 2009. More than 60 million tests have been performed since its authorization. In 2015, per Order of the Ministry of Health of Russia No. 951 of December 29, 2014, the RTA test replaced the Mantoux test in the screening of tuberculosis in children 8 years of age or older.

Studies have shown a significant increase in tuberculosis rates (37-fold in schoolchildren aged 8–17 years compared with the Mantoux test [12]) in primary healthcare centers following the transition to the screening using RTA.

The specificity of the RTA test was also evaluated in a local (Moscow) patient population with a low tuberculosis rate and a low risk of tuberculosis infection (contacts of pregnant women). In 2015–2016, 7,249 tests were performed in this population which most closely mirrors the permanent population of Moscow. A positive or equivocal RTA test result was found in 0.3%

(95% confidence interval [CI] 0.2–0.6%) and 1.0% (95% CI 0.7–1.4%) of evaluated pregnant contacts ($p < 0.05$) in 2015 and 2016, respectively. No tuberculosis patients were identified in this population. Thus, the specificity of the test was 99.0–99.7% [13].

With decreasing incidence of tuberculosis in the Russian Federation, it seems relevant to confirm the specificity of the RTA test in a population of healthy subjects who are not at risk of tuberculosis (with a negative T-SPOT.TB assay result) in real clinical practice in the subjects of the Russian Federation with low rates of tuberculosis incidence and mortality.

It should be noted that specificity, which is one of the key characteristics of a diagnostic test, is defined as the ability of a test to correctly identify subjects without the disease.

7. STUDY OBJECTIVES

7.1 Primary objective

To evaluate the specificity of the test with recombinant tuberculosis allergen (RTA) following intradermal administration in healthy subjects 18 to 30 years of age, not belonging to the risk group for the development of tuberculosis in the regions of the Russian Federation with a favorable epidemiological situation for tuberculosis.

The specificity of the RTA test is defined as the relative frequency of subjects in a healthy population (individuals with no signs of tuberculosis infection) who have a negative test reaction (no infiltration/papule) at the injection site).

7.2 Secondary objectives

To record all adverse events that occurred within 28 days after the test

7.3 Primary endpoint

- Absolute number and percentage of the volunteers with negative and positive test results with RTA (individuals with no signs of tuberculosis infection) based on the results of the assessment (measurement) of the infiltrate 72 hours after the test.

7.4 Secondary endpoints

- Local (at the injection site) adverse events (itching, swelling, soreness, pain) within 72 hours after administration of RTA.
- General and local adverse reactions within 28 days after administration of RTA.

7.5 Primary variables:

Diameter of the infiltrate (papule) at the injection site, measured across the longitudinal axis of the forearm 72 hours after the intradermal test.

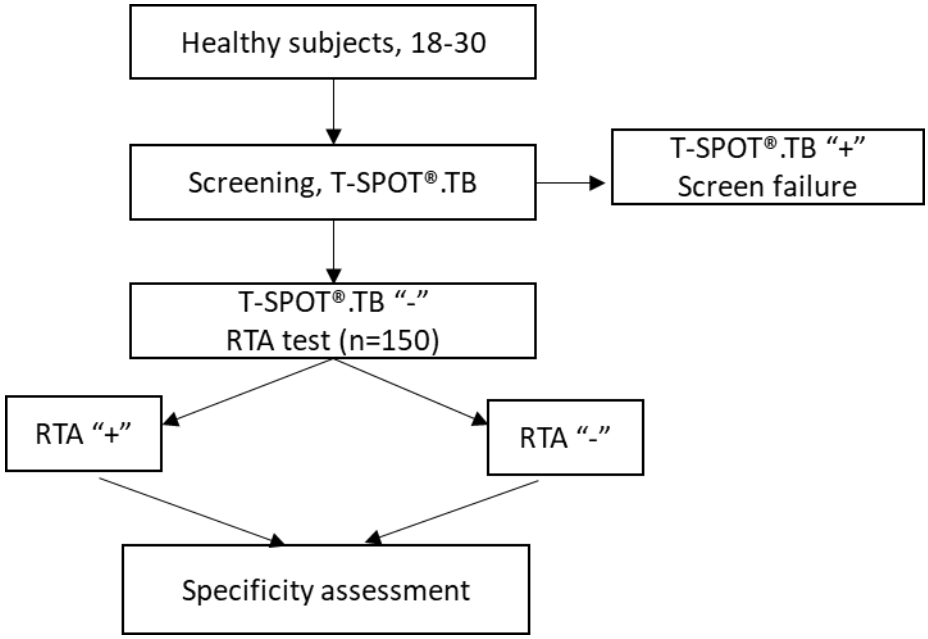
7.6 Secondary variables:

- Adverse events at the injection site within 28 days after the test
- All adverse events that occurred within 28 days after the test

8. STUDY DESIGN

8.1 Design

This clinical study is a prospective, multicenter, open-label study with the formation of a cohort of healthy adults (18-30 years of age) (not classified as at risk of tuberculosis, with no clinical symptoms of the disease, with a negative T-SPOT.TB test results) to evaluate the specificity of the test with recombinant tuberculosis allergen following administration of the investigational diagnostic product to each participant at a dose of 0.2 μg/0.1 mL. Study design is presented below (picture 1).



Picture 1. Study design

In this study, in accordance with the current national standards for diagnosis of tuberculosis in Russia, volunteers will undergo a comprehensive examination with an assessment of clinical manifestations of the disease (general and respiratory complaints) and an assessment of the objective status. Blood sampling for the T-SPOT.TB test will be performed before the test with recombinant tuberculosis allergen.

Volunteers' complaints, as well as adverse events that occurred during the follow-up period, will be recorded at all visits.

The main goal of this study is to evaluate the specificity of the skin test with recombinant tuberculosis allergen, defined as the relative frequency of participants in a population of healthy volunteers (with no signs of tuberculosis infection) whose test results are comparable to that of the T-SPOT.TB reference test.

Visit 0 *Screening visit* is for an initial examination, in accordance with generally accepted principles of diagnosis and monitoring, which includes:

- obtaining the informed consent
- assignment of an individual volunteer number
- completion of the questionnaire on inclusion/non-inclusion criteria
- collection of the volunteer's medical history, allergic history, complaints
- collection of information on the inclusion/non-inclusion criteria
- physical examination, including measurement of height and weight
- measurement of heart rate, breath rate, blood pressure
- blood sampling for the T-SPOT.TB test.

Visit 1 *A skin test injection visit (0-3 days post-screening) includes:*

- intradermal test with recombinant tuberculosis allergen in accordance with the regulatory documents in force in the Russian Federation in case of negative T-SPOT.TB test

- assessment of adverse events that occurred after the injection of the recombinant tuberculosis allergen.

Visit 2 *An assessment visit (after 72 hours)* includes*:*

- assessment of the reaction to the intradermal test with recombinant tuberculosis allergen.
- assessment of adverse events that occurred after the injection of the recombinant tuberculosis allergen.

Visit 3 *Follow-up Visit (28 days after Visit 1):*

- assessment of adverse events that occurred after the injection of the recombinant tuberculosis allergen.

The volunteer can enter screening and participate in the study only after signing the informed consent. After signing the informed consent the volunteer's compliance with the inclusion / non-inclusion criteria is assessed If a volunteer meets at least one non-inclusion criterion, he/she will not be able to continue his/her participation in the study.

The T-SPOT.TB test will be performed at Visit 0 prior to the test with recombinant tuberculosis allergen as a reference method for diagnosis of tuberculosis infection.

If the result of the T-SPOT.TB test is negative and other inclusion criteria are met, the volunteer will be enrolled in the study and can continue his/her participation in the study (the study design is presented in Appendix 3).

For practical reasons, Visit 1 (product administration) should be scheduled for Monday, Tuesday, or Friday (to allow 72 hours follow-up after RTA administration). See section 9.3 for more information about visits.

The occurrence of an infiltrate of any size at the RTA injection site will be regarded as a positive result.

The T-SPOT.TB test results will be regarded as negative, indeterminate, or positive upon receiving results from the laboratory performing the test in accordance with the Instructions for use. If the result of the T-SPOT.TB test is indeterminate or positive, the volunteer will be excluded from further participation in the study.

All results will be recorded in the individual Case Report Form (CRFs) of the study participants.

Healthcare professionals will ensure that informed consent is obtained from the volunteers for the skin test and blood collection for research purposes.

Each volunteer participating in the study will be monitored throughout the study. Anonymized CRFs will be shared between the study sites by courier delivery or their copies via e-mail.

A CRF for each visit is provided in Appendix 4.

8.2 Study population and clinical study conduct

Healthy individuals 18 to 30 years of age, not belonging to the risk group for the development of tuberculosis in the regions of the Russian Federation with a favorable epidemiological situation for tuberculosis.

8.3 Number of study subjects

150 healthy BCG-vaccinated male and female volunteers 18 to 30 years of age.

8.4 Subject recruitment

Volunteers will be recruited by the centers included in the study. They will comply with all inclusion criteria. Recruitment will be performed via announcements.

8.5 Inclusion criteria

1. Signed informed consent for the participation in the study, as well as for the Investigator's access to medical records.
2. Age 18 to 30 years
3. A history of BCG vaccination (confirmed by medical documentation and/or the presence of BCG scar)
4. Healthy individual according to physical examination and medical records at screening.
5. Willingness to cooperate and follow the recommendations of the Investigator in accordance with the Protocol.

8.6 Non-inclusion criteria

Subjects will not be enrolled in the study for any of the following reasons:

1. A history of tuberculosis or close contact with a patient with active tuberculosis for 5 years prior to the enrollment in the study.
2. Positive T-SPOT.TB test at the enrollment in the study
3. Treatment with drugs affecting immune system within 3 months prior to the enrollment in the study
4. Vaccination against any infections <1.5 months prior to the enrollment in the study
5. Vaccination with BCG <6 months prior to the enrollment in the study.
6. The Mantoux test with 2 TU and/or the test with the recombinant tuberculosis allergen was performed less than 6 months prior to the enrollment in the study.
7. Congenital or acquired immunodeficiency.
8. Active disease of the immune system
9. HIV infection.
10. The current condition of the skin interferes with the conduct and reading of skin tests (trauma, skin diseases).
11. A disease in which blood sampling poses a risk to the volunteer (hemophilia, other bleeding disorders) or obstructed venous access.
12. The volunteer currently participates in another clinical study or has participated in another clinical study within 3 months prior to the enrollment in the study.
13. Previous participation in clinical studies of ESAT-6 and/or CFP-10 antigens.
14. Pregnancy, lactation, pregnancy planning.
15. The reluctance of a female person to use effective barrier (including spermicidal gel), hormonal or intrauterine contraceptives during the study.
16. History of alcohol, drug, benzodiazepine, or other substance abuse within the 12 months prior to the enrollment in the study.
17. Use of alcoholic beverages within 24 hours prior to the visit.
18. A condition or disease that, in the opinion of the Investigator, is inappropriate for participation in the study.

8.7 Exclusion criteria

1. Refusal to participate in the study.

8.8 Anticipated reasons for study discontinuation

Volunteers may discontinue participation in the study at any time without giving a reason.

No medical events are foreseen that might cause a volunteer to drop out of the study, since invasive procedures are only performed during Visit 0 and Visit 1. Procedures performed at subsequent visits do not expose the study participant to any risk..

The study may be stopped at any time if the Principal Investigator concludes that participation in the study exposes the volunteers to unacceptable risks.

If for any reason a volunteer wishes to withdraw from the study or has to be withdrawn from the study at the discretion of the Principal Investigator, the date and reason for the withdrawal should be documented in the CRF.

8.8.1 Permanent contraindications

Permanent contraindications are listed as the non-inclusion criteria in Section 8.6.

8.8.2 Temporary contraindications

The Responsible Investigator may, at his or her discretion, decide to postpone a visit based on the health assessment findings of a subject on the scheduled testing day.

9. INVESTIGATIONAL MEDICINAL PRODUCT

Investigational medicinal product:

Generic name: Standard dilution of recombinant tuberculosis allergen

Trade name: Diaskintest

Manufacturer: GENERIUM JSC, Russian Federation

Dosage form: solution for intradermal injection.

Appearance: clear, colorless liquid

One dose of the product is 0.1 mL/0.2 µg

ATC code: V01AA20

9.1 Composition

Investigational medicinal product: Recombinant tuberculosis allergen (Diaskintest).

Recombinant protein CFP10-ESAT6 …….0.2 µg

Sodium phosphate dibasic dihydrate ……..0.3876 mg

Sodium chloride …… 0.46 mg

Potassium phosphate monobasic……. 0.063 mg

Polysorbate 80 …… 0.005 mg

Phenol …….. 0.25 mg

Water for injection ……... q.s. to 0.1 mL

9.2 Procedure

The RTA intradermal test is performed according to the current prescribing information for the medicinal product. An RTA dose of 0.1 mL is injected into the inner surface on the mid-third of the right or left forearm. The injection is performed using a 1-mL syringe with a short beveled needle (a tuberculin syringe).

A qualified healthcare professional will read the result of the skin test (diameter of hyperemia/infiltrate (papule) in mm) 72 hours after the RTA injection. An infiltrate (papule) of any size will be considered a positive result.

9.2.1 Dose and administration

The product for the skin test is administered as per the prescribing information for RTA by healthcare professionals who have a certificate to perform the RTA test, appropriate training, and experience with the test. Blood samples will be collected by a staff member who is authorized, trained, and experienced in blood collection for the T-SPOT.TB assay.

The number assigned to the study subject should match the subject identification number on the package of the investigational medicinal product.

Administration of the product for the skin RTA test:

- The investigational medicinal product should be removed from the refrigerator half an hour before administration.
- The expiry date should be checked before administration.

- The investigational medicinal product may be administered only if it has the appearance of a clear and colorless solution.
- Injection site disinfection is not required before administration. If the injection site does get disinfected, it must be allowed to dry completely before administration of the investigational medicinal product.
- At Visit 1, the Investigator documents in which forearm the investigational medicinal product should be injected in the CRF.
- As per the RTA prescribing information, a dose of 0.1 mL should be administered using a 1.0-mL sterile disposable syringe with a 26-gauge short-bevel needle.
- The skin is slightly stretched, and the needle is held almost parallel to the skin, with the bevel facing upward. The needle should be inserted about 2 mm deep into the inner surface on the mid-third of the left or right forearm. The needle should be visible through the epidermis, and 0.1 mL of the investigational medicinal product is slowly injected intradermally. When placed correctly, the injection should produce a small papule on the skin, 8 to 10 mm in diameter. The papule will resolve in about 10 minutes.

The absence of a papule at the time of the intradermal RTA injection (as described above) should be documented in the CRF, providing a reason. Repeated administration of RTA to the same volunteer is not allowed. The volunteer should come to the follow-up visits as part of the study for safety monitoring. Volunteers without a papule after the intradermal injection of RTA are excluded from the analysis.

After injection, the syringe and needle should be disposed of as a unit in a labeled puncture-resistant container.

9.2.2 Packaging and labeling

The T-SPOT.TB assay and the RTA test are used routinely in specialized facilities, with all packaging requirements in place.

9.2.3 Transportation of the investigational medicinal products

The T-SPOT.TB assay and the RTA test are used routinely in specialized facilities. Transportation of the investigational medicinal product is not applicable in this study.

9.2.4 Storage conditions

The shelf life of RTA is 24 months when stored in a refrigerator at +2 °C to +8 °C, which will be monitored during the study.

Vials should be stored in cartons until the administration of the product. The expiry date is printed on the outer label of the carton.

At each study site, the investigational medicinal product should be stored in a refrigerator with a temperature control system and an alarm system.

In case of malfunction of the refrigerator storing the investigational medicinal product, it should be placed in another refrigerator as soon as possible. The reason and time when the investigational medicinal product was transferred to another place should be documented in a temperature control log.

The product must not be used if the Principal Investigator has found significant deviations from the recommended storage conditions. The Principal Investigator should discuss with the Clinical Study Leader whether the product should be disposed of or can be used in the study.

Monitoring of storage conditions shall be the responsibility of the study staff.

9.2.5 Procedure for generating a study participant number

On the day of screening, volunteers arriving at the center will be numbered consecutively (the first participant arriving will receive the lowest screening number). The individual volunteer number will be recorded in the informed consent and will be recorded in the individual sign-in cards at the visits thereafter.

The screening number is the individual volunteer number.

Method of generating a study participant number:

Each volunteer will be assigned the lowest available number during visit 0.

The available numbers in the study sites will be generated as follows:

The first digit is the first letter of the city where the study site is located;

The second to fourth digits are the smallest available three-digit number starting from 001.

9.2.6 Blinding и unblinding of data

This is an open-label study, and, hence, the study staff and subjects will be aware of the study stage and the products being administered. Subjects' data will only be present in informed consent forms which will not be shared or processed during the study, including all visits. Only study subject numbers will be available when the data are processed.

9.2.7 Compliance with the administration regimen of RTA and T-SPOT.TB

All procedures will be performed by the study staff during visits in a controlled environment. Monitoring of compliance with the administration regimen is not applicable in this clinical study.

9.2.8 Accounting for the investigational medicinal products

The T-SPOT.TB assay and the RTA test are used in routine practice. Product accounting beyond that is available at a given study site is not applicable.

9.2.9 Precautions and overdosage

As per the RTA prescribing information, transient signs of a general reaction, such as malaise, headache, and pyrexia, may rarely occur. These reactions, if any, will be documented in the CRF. No anaphylactic reactions associated with the tuberculin test have been observed. However, the necessary means to manage an anaphylactic reaction should always be available at the study site. The skin tests will be performed as per the current Russian guidelines for the diagnosis of tuberculosis which stipulate that these tests should be performed annually in certain subpopulations.

Venous blood draws may be associated with a minimal risk which could not jeopardize life or health.

9.2.10 Concomitant medication therapy

During the study, information on concomitant medication therapy considered relevant for the subject should be recorded on the appropriate pages of his/her CRF.

A decision to withdraw a subject from the study will be at the discretion of the Responsible Investigator. Drugs and live vaccines listed in Section 8.6 are among the non-inclusion criteria.

9.3 Study activities

Visit number	0	1	2	3
Study days	Day -3 to 0	0	3	28
Informed consent procedure - assignment of a study participant number	×			
Collection of the information relevant to the inclusion/non-inclusion criteria	×			
Medical history, symptoms	×	×	×	×
Medical examination	×	×	×	
Blood sampling for the T-SPOT.TB assay	×			
The RTA skin test (0.2 μg)		×		
Documentation of AEs		×	×	×
Reading of the RTA intradermal test result			×	

1) A Screening Visit (Visit 0) is performed within 3 days before an Enrollment Visit (Visit 1)

2) A Final Visit is performed if the subject discontinues the study before Visit 3 (Day 28).

3) The medical examination which includes a review of complaints and assessment of clinical symptoms is performed per the current guidelines. The examination must include measurements of body weight, height (only at screening), and body temperature, BP, HR, RR (performed according to the regulations adopted in Russia).

9.3.1 Assessment of specificity

Specificity is a key characteristic of a diagnostic assay, referring to its ability to correctly identify individuals without the disease.

The specificity of the RTA test is defined as the proportion of subjects who tested negative (no infiltration (papule) in the injection site) in a healthy population (i.e. without MTb exposure).

$$\text{Specificity} = \frac{\text{True negatives}}{\text{True negatives} + \text{False positives}}$$

9.3.2 Safety assessments

Local and systemic AEs will be evaluated based on observations made during the first hour after the administration of the investigational medicinal product and on health assessments performed

at follow-up visits on Days 3 and 28 after the administration of the investigational medicinal product. Medical examinations will be performed during all visits.

The Investigator will assess all AEs and will also report any serious AEs as described in Section 11.3. Any local or systemic AEs should be documented in the CRF.

All volunteers should be monitored by experienced medical staff at the study site for one hour after the interventions. Immediate-onset AEs, if any, should be documented in the CRF.

The intolerance assessment criteria include the frequency of AEs that occurred during the follow-up period.

Note: values obtained and documented at Visit 0 will be considered baseline.

9.3.3 Collection and handling of blood samples

Blood samples will be collected by venipuncture performed by trained healthcare professionals as per the standard protocols for medical manipulations.

Blood samples for the T-SPOT.TB assay

The T-SPOT.TB assay is used, stored, and analyzed according to the manufacturer's manual.

Blood is drawn from a vein into heparinized tubes (with a green cap). The first portion of blood should go into the heparinized tube. The volume of blood to be drawn for the study is 8 mL (one 8-mL tube or two 4-mL tubes). Immediately after drawing of the blood sample, the tube should be carefully turned upside down 3–4 times to prevent foaming and without shaking so that the blood is evenly mixed with the anticoagulant. The tubes are transported to the laboratory in the upright position at a temperature between +18 ºC and +25 C in a place protected from direct sunlight. The tubes must not be refrigerated or frozen. Each tube must be labeled with the full name (or the individual number of the subject), date, and time of blood collection. The accompanying form must clearly and legibly state the unabbreviated full name (or the individual number of the subject), date of birth, time and date when the material was collected. Non-compliance to the recommendations for the timeframe and temperature for storage and shipping, errors in the accompanying form and tube labeling may result in erroneous or invalid test results.

The T-SPOT.TB assay will be performed at certified laboratories in the participating regions. Subjects' numbers will be used as identifiers to ensure their confidentiality.

9.3.4 Schedule

An application to authorize the study is submitted to the local ethics committee (LEC) of the NMRC TPID in Q4 2021.

The date of the first visit of the first volunteer (First Volunteer In) is Q4 2021.

The date of the last visit of the last volunteer (Last Volunteer Out) is Q1 2022.

10. ETHICAL ASPECTS

Informed consent should be obtained from each volunteer as per local requirements after he or she has been informed of the nature of this study. Data collection and processing will be performed in compliance with all confidentiality principles.

All study participants will be examined in accordance with normative documentation in force in the Russian Federation. Volunteers will be informed that their consent to participate in the study is voluntary and that they may withdraw it at any time, without stating the reason or being sanctioned.

Approval will be obtained from the LEC of the NMRC TPID before the study commencement.

The total amount of blood to be drawn from each volunteer is approximately 8 mL. Blood samples will be coded before being sent to the laboratory so that only the Principal Investigator and study staff have access to the information linking the laboratory results to the individual volunteer number.

Volunteers should not expect any benefit from participating in the study, except for the benefit of a detailed health assessment to be conducted during the study.

10.1 Remuneration of subjects

Volunteers will not be paid for participation in the study.

10.2 Independent Safety Data Monitoring Committee

Subjects are expected to be exposed to only minimal risks during the clinical study. The Principal Investigator and Responsible Investigator of the study site shall be responsible for the safety of subjects in daily clinical practice. A singe fixed dose of the investigational medicinal product RTA will be administered to each subject during the study. Because the study has the design described above, an independent safety data monitoring committee is not required.

11. ADVERSE EVENTS

This section discusses the procedures for documenting and reporting AEs during the study. Relevant definitions and terms are presented. Serious AEs should be immediately reported to the NMRC TPID.

11.1 Definitions and terms

An adverse event (AE) is any unfavorable medical event detected in a patient or study participant after intradermal administration of the diagnostic test product and blood sampling, which may not have a causal relationship with its use. Thus, an adverse event (AE) can be any unfavorable symptom (including abnormal laboratory findings), complaint, or disease, the occurrence of which does not exclude a causal relationship with the use of the medicinal (investigational) product, regardless of the causal relationship with the medicinal product [ICH GCP E6(R2)].

A serious AE (experience) or reaction is any adverse medical event that is, regardless of the dose:

* results in death,

* is life-threatening,

NOTE: the term “life-threatening” in the definition of “serious” means an event during which the volunteer was under threat of death; it does not concern an event that could theoretically lead to death, was it more severe.

* requires hospitalization or prolongation of hospitalization,

* leads to persistent or significant incapacity/disability or

* is a congenital abnormality/malformation.

- Adverse events should be recorded in the CRF using medical terminology.
- Adverse events will be reported in accordance with local regulations.

11.2 Conventional adverse events reporting

The Investigator shall be responsible for documenting in CRFs all AEs that were reported at each visit. The Investigator should use the following terms:

The causal relationship between an AE and the study tests is assessed using the following terms:

- Unrelated
- Possibly related
- Probably related
- Definitely related

The severity of an AE is assessed using the following terms:

- Mild (i.e. tolerated easily)
- Moderate (i.e. interfering with day-to-day activities)
- Severe (i.e. precluding normal functioning)

The outcome of an AE is assessed using the following terms:

- Death
- Unresolved to date
- Resolved with sequelae
- Resolved without sequelae
- Unknown

Note. If an AE persists at the last visit, the Investigator should monitor it until it resolves or stabilizes.

The seriousness of an AE is assessed based on answers to the following questions:

- Did the event lead to death?
- Was the event life-threatening?
- Did the event require inpatient admission or prolongation of existing hospitalization?
- Did the event result in severe or permanent disability or incapacity?
- Does the event appear to be medically relevant?

To avoid confusion or misunderstanding of the difference between the terms “serious” and “severe,” which are not synonymous, the following explanatory note is provided:

The term “severe” is often used to describe the intensity (severity) of a specific event (e.g. mild, moderate, or severe myocardial infarction). However, the event itself may have relatively little medical significance (e.g., severe headache). The meaning of this definition is different from that of the term “serious,” which is based on patient/event outcomes or action criteria typically associated with events that are life-threatening or jeopardize the functioning of the subject. Seriousness (rather than severity) serves as a benchmark for determining regulatory reporting requirements.

11.3 Immediate reporting of serious adverse events by the Investigator

The Investigator documents a serious AE (see definitions in Section 11.1) in the CRF and is responsible for completing the serious AE reporting form (see a template in Appendix 5).

Any serious clinical AE (including death and overdose) that occurred during the study, regardless of the manipulations and procedures received by the volunteer, should be reported to the NMRC TPID within one working day after the serious AE occurred.

The completed Suspicious Adverse Reaction Reporting Form (Appendix 5) should be sent by confidential mail to the following address of the Ministry of Health of Russia: ul. Dostoevskogo 4, korp. 2, 127473 Moscow.

If important additional information is discovered after the initial report, subsequent reports should be sent (using the same form).

11.4 Expedited reporting of suspected unexpected serious adverse events by the Principal Investigator to the LEC

Deadlines for notifying the LEC of a suspected unexpected serious adverse reaction (SUSAR) after the initial information about the SUSAR was received:

- For a SUSAR that resulted in death or a life-threatening condition, a notification should be submitted within seven (7) calendar days after the SUSAR became known to the NMRC TPID; the report should be submitted in additional eight (8) calendar days
- For other SUSARs that are reported within fifteen (15) calendar days after the SUSAR became known to the LEC (prior notification is not required)

The Safety Report Form for reporting a suspected adverse reaction must be used in SUSAR reports.

12. DATA MANAGEMENT AND STATISTICAL ANALYSIS

12.1. General provisions

Data on the proportion of subjects who tested negative in a population of healthy subjects who are not at risk of tuberculosis are of key importance in the development of the RTA test.

The obtained results will allow us to conclude about the significance of the RTA test in the detection of tuberculosis infection.

The NMRC TPID shall be responsible for the management of the clinical study and statistical analysis of data received from all study sites.

12.2 Data management

Clinical data are collected in the CRF. The original CRFs remain in the custody of the study site. The NMRC TPID will receive copies of CRFs completed at each visit from the Responsible Investigators.

The Investigator shall be responsible for proper documentation of data in the CRF of each subject and related records. The Investigator signing the protocol signature page shall personally sign the CRF to ensure that observations and findings are correctly and completely documented in the CRF. Copies of CRFs should be submitted to the NMRC TPID on time and will be kept by the Principal Investigator in a designated storage area. Investigators will be requested to clarify any ambiguous or implausible data if any.

12.3 Analyzed populations

The population for the safety analysis includes all participants who had one RTA test during the study.

The efficacy population will be equivalent to the per-protocol population and will comprise all participants who have met the inclusion criteria.

12.4 Statistical methods

The study involves the analysis of anonymized CRF data that will be performed using an MS Excel spreadsheet:

- Study subject number;
- Enrollment date;
- Sex;
- Date of birth;
- BCG vaccination status (presence of a scar);
- Height;
- Body weight;
- Complaints;
- Body temperature;
- RR per minute;
- HR;
- BP (mmHg);

- Lung auscultation: rhonchi or crackles;
- The RTA test result (mm);
- The T-SPOT.TB assay result.

Generally accepted statistical methods will be employed to analyze the study data using Microsoft® Excel® 2016 MSO application software package (version 2109 16.0.14430.20154, 32-bit, product code: 00333-59091-97459-AA884). The use of the software is planned for statistical analysis. Two-sided statistical tests at a statistical significance level of $p < 0.05$ will be used.

Demographic and other baseline characteristics will be presented for all patients enrolled in the study (the full analysis set population).

The Shapiro-Wilk criterion will be used to determine the normality of the distribution. Parameters that are quantitative variables (including age) will be expressed as:

- Arithmetic mean (mean);
- 95% CI for the mean;

Qualitative and categorical variables (gender, etc.) will be expressed in absolute values (n/N) and proportions (%).

The study will evaluate the specificity of the RTA test (see. Paragraph 9.3.1).

12.5 Sample size determination

The sample size to assess the test specificity was initially estimated as the relative frequency in a binomial distribution.

For N patients, the variance of the estimated specificity is calculated as Var (û) = u (1–u)/N, where u is (unknown) the true specificity. For a fixed value N Var (û) reaches its maximum at u = 0.5. With N = 150, this maximum is 0.25/N = 0.0017, which corresponds to standard deviation (SD) (û) = √(Var (û)) = 0.041. Since true specificity is expected to be higher than 0.5, an SD of 0.041 would be the worst-case scenario. With a more realistic value of true specificity of 0.9 (specificity in studies to assess the specificity of RTA was >90%), the corresponding SD would be 0.024 which is considered an acceptable estimated accuracy.

12.6 Interim analysis

No interim statistical analysis is anticipated.

13. PROVISIONS OF THE GOOD CLINICAL PRACTICE GUIDELINES

This protocol has been written in compliance with the ICH guidelines, Section E6 of the ICH and Section E2A of the ICH, the EU directives 2001/20/EC and 2005/28/EC of the European Parliament (including the relevant guidance), and the National Standard of the Russian Federation GOST R 52379-2005 "Good Clinical Practice."

The study will be conducted in compliance with the principles of the Declaration of Helsinki (Appendix 6).

13.1 Study Participant Information and Informed Consent Procedure

The subjects are informed about the study verbally and in writing, with clear explanations regarding the procedures, potential risks, and benefits of the study. The subjects will be given sufficient time (at least 24 hours) to decide whether to participate in the study.

The informed consent must be signed and dated by the subject. A volunteer can be screened (Visit 0) and participate in the study only after signing informed consent.

The written information and informed consent form shall be approved by the relevant LEC before administration.

13.2 Submission and approval of the application to the LEC

The application file will be submitted to the LEC of the NMRC TPID for approval. Enrollment of subjects will not begin until the study has been approved by this authority.

The NMRC TPID is responsible for submitting the study application to the LEC.

13.3 Protection of personal data of study subjects

The Responsible Investigator of the study site shall be responsible for assigning an individual number to a volunteer after he or she signed the voluntary informed consent. Screening failures should be documented in the Visit 0 CRF, including reasons for exclusion or non-inclusion.

Voluntary informed consents and the Visits 0–3 CRFs must be signed by the Responsible Investigator of the study site to verify the accuracy of the entries.

Only the individual participant number must be included in the CRF. A CRF should be always identifiable by the respective voluntary informed consent.

Subjects should be aware of and agree to the following information:

- After the end of the clinical study (i.e. after the Last Volunteer Out date), the Principal Investigator will be keeping the voluntary informed consent forms and Visit 0–3 CRFs for at least 15 years.
- After the end of the clinical study, the voluntary informed consent forms and Visit 0–3 CRFs should be available to the LEC or other regulatory authorities for at least 15 years, if required for the inspection.

13.4 Investigator responsibilities

By signing this protocol, the Principal Investigator and the Responsible Investigator(s) of study sites shall bear overall responsibility to perform the clinical study in compliance with the protocol, GCP principles, and other relevant national normative documentation to ensure complete and accurate documenting of all data, including all AEs documented in CRFs, and to notify the NMRC

TPID immediately about serious AEs as per the procedures described in Section 11 of this protocol.

13.5 Curriculum vitae and registration of study staff

The NMRC TPID must have up-to-date, dated, and signed Russian versions of CVs of the Clinical Study Leader, Responsible Investigators, etc., involved in the study.

13.6 Training

The Principal Investigator shall bear overall responsibility to ensure that the study staff in all study sites has appropriate qualifications before the study commencement.

The Clinical Study Leader shall bear responsibility to provide training courses on general GCP issues, such as safety procedures, reporting of suspected serious adverse reactions including SUSARs, informing subjects, creating CRF entries, handling of diagnostic investigational medicinal products, randomization procedures, blinding and unblinding procedures, etc. If necessary, Responsible Investigators of the study site provide training sessions on the RTA test technique, etc.

13.7 Monitoring

The Responsible Investigator from the NMRC TPID will oversee the conduct of the clinical study in the study sites. Scope of inspection includes:

- Compliance with protocol requirements
- Appropriate resources, equipment, and staff
- Accuracy of the completion of CRFs
- Concordance between the CRF and source data
- Proper storage conditions of the product at the study site
- Proper storage conditions of the Investigator's File at the study site

Any issue is discussed and resolved with the Investigator or designated study staff. The oversight of the clinical study will be performed as per the GCP principles.

13.8 Audit and inspection

The NMRC TPID has ethical, legal, and scientific obligations to oversee the conduct of the study per the protocol and GCP standards.

The NMRC TPID may perform an audit of the study site. The audit will include monitoring of the administration of products, checking the availability of necessary documents, checking the procedure of signing the Information Sheet of the Informed Consent Form, and checking the accuracy of the completion of CRFs.

The date and time of the audit shall be agreed upon with the Investigator involved.

The Investigator agrees that the NMRC TPID will monitor the proper conduct and documentation of the study during routine visits. Data irrelevant to the study should be deleted or made unrecognizable.

The Investigator consents to an audit of the study site by an auditor designated by the NMRC TPID, as well as by a representative of the relevant authorities and ethics committees to confirm

that the conduct and documentation of the study comply with the study protocol, ICH-GCP regulations, and applicable law.

The Auditor must analyze all essential documents and verify that a randomly selected sample of data entered in the CRFs concurs with source/primary data of clinical charts. Thus, the Investigator grants direct access to source data/documents and premises where the study-related procedures are performed. It may be necessary that the Investigator be present during the monitoring and audit procedures for several hours.

13.9 Definition and archiving of source data

Source data are defined as all information in the original records and validated copies of the original records required to reproduce and evaluate a clinical study. The original documents are kept at the study sites. Examples of source data:

- Documents approved by the LEC
- Signed informed consent forms
- Printed results of laboratory testing
- CRFs (data entered directly into the CRF)
- Consent of the study staff to participate in the study

Copies of anonymized CRFs will be sent to the NMRC TPID by courier delivery or e-mail.

13.10 Definition and archiving of essential documents

The Principal Investigator is responsible for keeping essential documents in accordance with the ICH guidelines for at least 15 years after the study completion. The end of this clinical study is defined as the Last Subject Out.

During the monitoring of the study site, the Responsible Investigator shall agree on and ensure compliance with the archiving requirements to complete the study.

14. CONFIDENTIALITY OF INFORMATION AND DISCLOSURE OF INTERESTS

All CRFs, information, and results obtained by the NMRC TPID, as well as other information, including applications for not previously published patents and manufacturing processes, are considered confidential and shall remain the exclusive property of the NMRC TPID.

An integrated statistical and clinical study report will be prepared by the NMRC TPID.

Without a written NMRC TPID approval, no clinical study data may be published, submitted, or shared, except to regulatory agencies or the LEC, before the release of an internal clinical study report.

All Investigators agree to avoid discussing third-party issues or publish any results of the study unless the NMRC TPID can provide comments.

In the case of publication of the study results in a scientific journal, the names and order of appearance of authors will be determined by the NMRC TPID.

15. PARTNERSHIP AGREEMENT

The partnership agreement between the NMRC TPID and each study site should be signed before the enrollment of the first subject in the clinical study and should clearly define the rights and obligations of the parties involved.

16. PROTOCOL AMENDMENTS

Clinical study procedures may be changed upon agreement with the Principal Investigator and the Clinical Study Leader. If the changes are substantive, the LEC approval should be obtained before their implementation. All substantive changes should be documented as protocol amendments, and a newer protocol version should be compiled as necessary.

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