T-SPOT.*TB*

Frequently asked **questions**

Oxford Immunotec

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References

Tuberculosis: definition, infection and disease

1

What is the scale of the tuberculosis (TB) problem?

Although effective treatment has been available for over 70 years, TB is the leading cause of death from an infectious disease worldwide. The World Health Organization (WHO) estimates that more than one-third of the world's population is infected with *Mycobacterium tuberculosis*. TB continues to be a significant disease due to factors such as immigration, the emergence of drug-resistant TB strains, HIV, and other conditions that weaken the immune system.

2

How is tuberculosis (TB) spread?

TB is passed from person to person through the air. Individuals with pulmonary (lung) TB can propel aerosols containing *Mycobacterium tuberculosis* complex organisms into the air when they cough, sneeze, sing, speak or spit. Persons who then inhale these aerosols can become infected. Factors that determine the probability of infection include the immune status of the exposed individual, infectiousness of the TB contact and the proximity, frequency and duration of exposure.

3

What is TB infection (Latent Tuberculosis Infection "LTBI" or "latent TB")?

Individuals with TB infection ("LTBI" or "latent TB") harbor dormant *Mycobacterium tuberculosis* complex organisms in their bodies but are not infectious and do not have symptoms of TB disease. TB infected individuals usually have a positive T-SPOT.*TB* test result; however, assessing the probability of infection requires a combination of epidemiological, historical, medical and diagnostic findings. It is estimated that 10% of immunocompetent persons with latent TB infection will develop TB disease during the course of their lives. Approximately half of these individuals will develop TB disease within the first two years after infection, while the other half are at risk of developing TB disease at some stage in their life. The risk of progressing from TB infection to TB disease is increased in those with a weakened immune system.

3

For example, patients living with HIV/AIDS are at a greatly increased risk of progressing to TB disease, as are patients who have undergone an organ transplant, or are receiving immunosuppressive therapy. When appropriately diagnosed, latent TB infection can be treated with antibiotics. Urgency of treatment is dictated by degree of risk of progression which is derived from consideration of epidemiological, medical and diagnostic findings.

4

What is TB disease ("active TB")?

TB disease, or active TB, develops when the immune system cannot prevent *Mycobacterium tuberculosis* complex organisms from multiplying in the body. After exposure, persons can develop latent TB infection or TB disease. TB disease most commonly occurs in the lungs (pulmonary TB) but may occur in other body organs or spaces, particularly in immunocompromised patients or children, and may be localized or disseminated as occurs in miliary TB. Symptoms of pulmonary TB disease may include fever, cough, night sweats, weight loss, and fatigue. Without treatment, TB mortality rates are high. Individuals with TB disease usually have a positive T-SPOT.*TB* test result; however, assessing the probability of disease requires a combination of epidemiological, historical, medical and diagnostic findings. The definitive diagnosis of TB disease is made on the basis of isolation and identification of the TB mycobacterium in culture. Identification of the mycobacterium by other means, such as genetic tests of its presence, are increasingly being accepted as proof of infection. Persons with latent

TB infection are also at risk for progression to TB disease, with that risk being modulated by age, concomitant illness, medication and other epidemiological factors.

5

Are certain groups of individuals at an increased risk of exposure to *Mycobacterium tuberculosis*?

Yes, certain groups are more likely to be exposed to *Mycobacterium tuberculosis*, which may lead to TB infection and/or disease. These include:

- known close contacts of a person with infectious TB disease, primarily pulmonary TB
- persons living in, immigrating from, or traveling to TB-endemic regions of the world
- persons who work or reside in facilities or settings with individuals who are at high risk for TB (e.g. hospitals, homeless shelters, correctional facilities, nursing homes, boarding schools, or residential facilities for persons living with HIV/AIDS)

6

Are certain individuals at an increased risk of progressing from latent TB infection to TB disease?

The risk of progression to TB disease is higher in certain individuals, including:

- Persons with weakened immune systems
- Children under the age of five
- Persons living with HIV/AIDS
- Organ or hematologic transplant recipients
- Persons with radiographic evidence of prior healed TB
- Those undergoing medical treatments with immunosuppressive agents such as systemic corticosteroids, TNF-α antagonists and therapies following transplantation
- Persons with leukemia or cancer of the lung, head or neck
- Cigarette smokers

- Drug or alcohol abusers
- Persons with a low body weight
- Persons with silicosis, diabetes mellitus or chronic renal failure/hemodialysis
- Persons who underwent gastrectomy or jejunoileal bypass
- Persons infected with TB within prior 2 years

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How important is treatment for TB disease?

Treatment for TB disease is vital. The goals of treatment include not only curing the patient, but reducing transmission to others. In general, the duration of treatment is 6 – 9 months, but is much longer in those with drug-resistant TB. It is crucial that individuals with TB complete their entire course of treatment even if their symptoms improve. TB that is not adequately treated can reactivate or become resistant to drugs, making it more difficult to treat.

8

Why is the treatment period for TB disease so long?

Most antibiotics capable of destroying bacteria can only do so while the bacteria are actively replicating. The replication cycle of *Mycobacterium tuberculosis* complex organisms is relatively long; therefore, lengthy treatment is required to ensure that all of the bacteria are destroyed. If the treatment is inconsistent or too short, some bacteria may survive, potentially allowing tuberculosis (TB) disease to reactivate or develop drug-resistance.

9

How important is treatment for TB infection ("LTBI" or "latent TB")?

Treatment for latent TB infection is fundamental in preventing TB disease and overall TB elimination efforts. Completed treatment regimens reduce the risk of TB disease by up to 90%. Urgency of treatment is dictated by degree of risk of progression which is derived from consideration of epidemiological, medical and diagnostic findings.

In 2015, recognizing latent TB infection testing and treatment as critical components of ultimately eliminating TB disease, the World Health Organization (WHO) set forth its first guidelines on managing latent TB infection.

Visit: www.who.int/tb/publications/latent-tuberculosis-infection/en/

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TB Detection

Is there a test for the detection of TB infection ("LTBI" or "latent TB")?

- Tuberculin Skin Test (TST)
- Interferon-Gamma Release Assays (IGRAs)

There are several methods to detect TB infection, broadly divided into tuberculin skin tests and blood tests:

- A TST, which has been used to detect TB infection for over 100 years, requires an intradermal injection of a small amount of purified protein derivative (PPD) into the skin. In 48-72 hours, the resultant induration is measured.

- More recently, blood-based tests, referred to as interferon-gamma release assays (IGRAs), have been introduced. The technology of the T-SPOT.*TB* test, an IGRA, is based on the release of interferon-gamma secreted by individual effector T cells (both CD4+ and CD8+) after being stimulated by TB-specific antigens.

11

Is there a test for the detection of TB disease ("active TB")?

Identification of individuals with active TB disease is critical to TB control. Those suspected of having TB disease may undergo a number of tests to confirm the diagnosis (e.g. chest x-ray, sputum culture, smear microscopy, PCR). Samples from the sputum or other sites are collected and cultured to categorically confirm the diagnosis of TB and to determine susceptibility of the strain to a range of antibiotics used for treatment. The T-SPOT.*TB* test may be used as a diagnostic aid in suspected TB disease patients when used in conjunction with radiography and other medical and diagnostic evaluations. The T-SPOT.*TB* test, like a TST and the ELISA-based TB blood test, detects TB infection but does not differentiate between active TB disease and latent TB infection.

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What are the limitations of a tuberculin skin test (TST)?

Limitations of a TST, as well as factors which can influence test performance and accuracy, include:

- Noncompliance
    - o Requires a minimum of 2 visits
    - o Failure to return for TST interpretation
- Subjective Results
    - o Misreading of induration
    - o Poor inter-observer reproducibility
    - o Cutoff dependent on individual risk factors
- False-Positive Results
    - o Cross-reactivity with BCG vaccine
    - o Cross-reactivity with non-tuberculous mycobacteria (NTM)
    - o Errors in TST placement or reading

- False-Negative Results
    - Anergy (inability to produce an immune response)
    - Immunocompromised status
        - HIV, cancer
    - Immunosuppression
        - Biologics, corticosteroids
    - Errors in TST placement or reading
- Costly
    - Inaccurate results
    - Solution waste
    - Staff must be trained in TST placement and interpretation

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BCG vaccination

What is Bacille Calmette-Guérin (BCG) vaccination?

The BCG vaccine is used in many countries with a high prevalence of TB to prevent childhood tuberculous meningitis and miliary disease, but confers limited protective value in adults. The BCG vaccine is also used as an immunotherapeutic agent for individuals with bladder cancer. BCG-vaccinated individuals may produce a positive TST, even if they are not infected with *Mycobacterium tuberculosis* complex organisms. This is a common cause of TST inaccuracy.

T-SPOT.*TB* Test description and performance

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What is the intended use of the T-SPOT.*TB* test?

The T-SPOT.*TB* test is an *in vitro* diagnostic test for the detection of effector T cells that respond to stimulation by *Mycobacterium tuberculosis* antigens ESAT-6 and CFP10 by capturing interferon-gamma in the vicinity of T cells in human whole blood collected in sodium citrate or sodium or lithium heparin. It is intended for use as an aid in the diagnosis of *M. tuberculosis* infection.

The T-SPOT.*TB* test is an indirect test for *M. tuberculosis* infection (including disease) and is intended for use in conjunction with risk assessment, radiography and other medical and diagnostic evaluations.

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Why does the T-SPOT.*TB* test measure interferon-gamma?

Interferon-gamma is crucial to immune defense against intracellular pathogens such as *Mycobacterium tuberculosis*. Post infection, naïve T cells become sensitized to TB-specific antigens and develop into TB-specific effector T cells (both CD4+ and CD8+), which then migrate to the site of infection and secrete interferon-gamma to activate macrophages to ingest and destroy mycobacteria. TB-infected patients have TB-specific effector T cells circulating in their peripheral blood, which secrete interferon-gamma *in vitro* when stimulated by the antigens used in the T-SPOT.*TB* test. The T-SPOT.*TB* test directly captures interferon-gamma secreted by individual TB-specific effector T cells.

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Does the T-SPOT.*TB* test differentiate between latent TB infection and TB disease?

No, like a tuberculin skin test (TST) and the ELISA-based TB blood test, the T-SPOT.*TB* test does not differentiate latent TB infection from TB disease.

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What data is there to support the T-SPOT.*TB* test in clinical use?

The body of scientific evidence demonstrating the performance of the T-SPOT.*TB* test for the detection of TB infection (latent and active) in various patient populations continues to grow. Hundreds of peer-reviewed publications, including a number of meta-analyses, have been published covering a wide range of clinical and epidemiological settings including:

- Latent TB infection ("LTBI")
- TB disease (including pulmonary and extra- pulmonary TB)
- HIV infected patients
- anti-TNF-α therapy candidates
- Healthcare worker screening
- Children, adults and the elderly
- Malnourished individuals
- Renal patients undergoing dialysis

- Patients with oncological disorders
- Low prevalence countries
- High prevalence countries
- TB contact tracing
- Transplant patients

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How soon after exposure to *Mycobacterium tuberculosis* can an infection be detected with the T-SPOT.*TB* test?

The time interval for conversion following exposure is not yet well defined, but is expected to occur no later than tuberculin skin test (TST) conversion (typically 2-8 weeks).

According to the core curriculum on tuberculosis set forth by the Centers for Disease Control and Prevention (CDC) it is recommended that contacts of a person with TB disease, who have a negative initial interferon-gamma release assay (IGRA) or TST within 8 weeks of exposure, be retested 8 - 10 weeks after last exposure.

REFERENCE: 2, 6, 9, 11

T-SPOT.*TB* Test performance characteristics

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What is the sensitivity and the specificity of the T-SPOT.*TB* test?

The sensitivity of the T-SPOT.*TB* test is 95.6% and the specificity of the T-SPOT.*TB* test is 97.1%.

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Why is sensitivity important in a test for *Mycobacterium tuberculosis* infection?

Sensitivity (the ability to detect TB-infected individuals) is one of the key measures of diagnostic performance. A test with high sensitivity, such as the T-SPOT.*TB* test, will have few false-negative results. This is especially important in patients that carry a greater risk of progression from latent TB infection to TB disease, such as those with weakened immune systems.

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Why is specificity important in a test for *Mycobacterium tuberculosis* infection?

In addition to sensitivity, specificity (the ability to identify non-infected individuals) is another key measure of diagnostic performance. A test with high specificity, such as the T-SPOT.*TB* test, will have few false-positive results, minimizing unnecessary follow-up diagnostic or therapeutic procedures.

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Can the T-SPOT.*TB* test be used in testing specimens from patients with weakened immune systems?

Yes, the T-SPOT.*TB* test is well-suited for use in patients with weakened immune systems. Each specimen undergoes a cell count which is used to create a normalized (standardized and known number) suspension of cells that are subsequently incubated with TB-specific antigens. Immunocompromised patients may have a reduced number of peripheral blood mononuclear cells (PBMCs) - the types of white blood cells used in the T-SPOT.*TB* test. In these patients, multiple blood tubes can be pooled to obtain the required number of cells to perform the test.

Pivotal clinical study data submitted to the US Food and Drug Administration (FDA) for pre-market approval extensively evaluated the T-SPOT.*TB* test in immunocompromised individuals including, but not limited to, subjects with HIV, silicosis, diabetes, end-stage renal disease and organ transplant. A negative tuberculin skin test was associated with being immunocompromised; in contrast, no association was observed between T-SPOT.*TB* test result and immunocompromised status.

23

Are any patient groups excluded from testing with the T-SPOT.*TB* test?

According to the ATS/IDSA/CDC 2016 guidelines, the T-SPOT.*TB* test is acceptable for all patient groups and preferred in situations where a patient is likely to be infected with a low to intermediate risk of progression. For those patients greater than 5 years of age who have a history of BCG vaccination or are unlikely to return to have their TST read, the IGRA is also preferred. A TST is preferable in healthy children under the age of 5, for whom testing is warranted, due to the limited data available in this age group. The American Academy of Pediatrics (AAP) also prefers a TST be used in patients under 5 years of age but acknowledge that some experts will use an IGRA in children 2 to 4 years of age, especially if they have received a BCG vaccine.

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Is a positive T-SPOT.*TB* test result expected in patients with a previous history of tuberculosis?

Unfortunately, there is no clear answer to this question. Studies do not consistently demonstrate that individuals test negative after TB treatment. Clinical cure is described by negative sputum culture, improvement of symptoms and chest x-ray changes. The term clinical cure refers to both a sterilizing cure and the return to the quiescent phase (latent TB infection). Some data have shown that a large proportion of individuals remain skin or blood test positive. The persistence of effector T cells, the cells stimulated by TB-specific antigens in the T-SPOT.*TB* test, may suggest the presence of dormant bacteria but further study is required. Depending on requirements, other diagnostic tests and medical examinations could be considered if the patient remains positive after treatment.

25

Are infections with mycobacteria other than *Mycobacterium tuberculosis* expected to produce positive T-SPOT.*TB* test results?

Mycobacterium tuberculosis is the causative agent of most cases of tuberculosis and thus, T-SPOT.*TB* test sensitivity was determined from subjects with active, culture-confirmed *Mycobacterium tuberculosis* infection. Individuals infected with other *Mycobacterium tuberculosis* complex organisms (such as *M. bovis, M. africanum, M. microti, M. canetti*) usually have T cells in their blood which recognize the antigens, ESAT-6 and CFP10, used in the T-SPOT.*TB* test and are also anticipated to produce positive T-SPOT.*TB* test results. ESAT-6 and CFP10 antigens are absent from most non-tuberculous mycobacteria (NTM) with the exception of *M. marinum, M. szulgai*

and *M. kansasii*. While it is unclear if ESAT-6 and CFP10 are present in the genome of all subspecies of *M. gordonae*, it is possible that this NTM may also produce a positive result. All other non-tuberculous mycobacteria, including *M. avium*, are not expected to cross-react with the antigens used in the T-SPOT.*TB* test.

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Can the T-SPOT.*TB* test detect infections of drug-resistant tuberculosis strains such as MDR-TB, XDR-TB or TDR-TB?

Yes, the T-SPOT.*TB* test can detect all strains of *Mycobacterium tuberculosis* complex organisms, including multi-drug-resistant tuberculosis (MDR-TB), extensively-drug-resistant tuberculosis (XDR-TB) and totally-drug-resistant tuberculosis (TDR-TB). The antigens in the T-SPOT.*TB* test are common to all *Mycobacterium tuberculosis* strains. However, the test does not predict whether the organism is sensitive to usual drug treatments. Drug susceptibility testing occurs after isolation and identification of the organism by other methods.

27

What regulatory approvals does the T-SPOT.*TB* test have?

The T-SPOT.*TB* test has carried the CE mark, which allows it to be marketed in the EU, since 2004. Additionally, the T-SPOT.*TB* test received US Food and Drug Administration (FDA) premarket approval in 2008, was approved in China in 2010 and in Japan in 2012. Approvals have also been obtained in many other countries, including, but not limited to, Canada, Taiwan, Russia, Singapore, Thailand, Peru, Nigeria and Mexico.

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Is the T-SPOT.*TB* test affected by previous BCG vaccination?

Unlike a tuberculin skin test, there is no association between BCG vaccination and T-SPOT.*TB* test results. The BCG vaccine is an attenuated derivative of virulent *Mycobacterium bovis*, the bovine or animal form of the TB mycobacterium. The T-SPOT.*TB* test utilizes antigens (ESAT-6 and CFP10) that are located on a genomic region designated as RD1, region of differentiation 1. The RD1 region is present in all virulent *M. bovis* strains but is deleted from all BCG strains. Because the antigens used in the T-SPOT.*TB* test are not present in the BCG vaccine, the T-SPOT.*TB* test does not produce a false-positive result due to BCG vaccination. It should be noted, however, that patients infected with virulent *M. bovis* are likely to produce a positive T-SPOT.*TB* result.

T-SPOT.*TB* advantages over tuberculin skin test (TST)

29

What are the advantages of the T-SPOT.*TB* test?

The T-SPOT.*TB* test is the only TB test with sensitivity and specificity exceeding 95% in pivotal clinical studies. The T-SPOT.*TB* test is reliable even in challenging testing populations, including BCG-vaccinated and immunosuppressed persons, and relies on routine phlebotomy procedures.

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What are the advantages of the T-SPOT.*TB* test over a tuberculin skin test (TST)?

The T-SPOT.*TB* test has a number of advantages over a TST, including:

- Single visit to complete test (versus 2 - 4 with TST)
- Improved sensitivity (reliable in patients with weakened immune systems)
- Improved specificity (not affected by BCG vaccine and most non-tuberculous mycobacteria)

31

Can the T-SPOT.*TB* test be used in place of a tuberculin skin test (TST)?

According to the ATS/CDC/IDSA 2016 guidelines, an interferon-gamma release assay (IGRA), such as the T-SPOT.*TB* test, is acceptable in all LTBI testing situations. An IGRA is preferred in situations where the patient is likely to be infected and has a low to intermediate risk of progression to disease, as well as in situations where the patient is unlikely to be infected but LTBI testing is required. IGRAs are also preferred in patients that are at least 5 years of age that are BCG-vaccinated or unlikely to return for their TST reading. Please refer to the full guidelines for complete information.

T-SPOT.*TB* test results

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How are T-SPOT.*TB* test results interpreted?

T-SPOT.*TB* test results are qualitative and are reported as positive, borderline (equivocal) or negative, given that the test controls perform as expected. Test results are determined by firstly enumerating the spots (captured interferon-gamma from individual T cells) in each of the patient's four test wells (Positive Control, Nil Control, Panel A, Panel B). Spots can be counted from the test wells using a magnifying glass, stereomicroscope, or a digital image captured from a microscope. Qualitative results are interpreted by subtracting the spot count in the Nil (Negative) Control from the spot count in Panels A and B. Detailed information regarding test result interpretation can be found in the T-SPOT.*TB* package insert.

33

How are T-SPOT.*TB* test results reported?

T-SPOT.*TB* test results are reported as positive, negative, borderline, or invalid.

34

What action should be taken if the T-SPOT.*TB* test is positive?

Patients testing positive with the T-SPOT.*TB* test likely have TB infection and should be clinically evaluated for active TB disease. Risk of one or the other can be assessed on the basis of a combination of epidemiological, historical, medical and diagnostic findings. A definitive diagnosis of active TB disease is made on isolation and identification of the mycobacterium from the patient.

35

What is a T-SPOT.*TB* borderline result?

The vast majority of T-SPOT.*TB* test results are either positive or negative. A small percentage of test results can be borderline (equivocal), where the higher of (Panel A minus Nil Control) and (Panel B minus Nil Control) is 5, 6 or 7 spots. The borderline category is intended to reduce the likelihood of false-positive or false-negative results around the cutoff point of the T-SPOT.*TB* test. As opposed to an indeterminate or invalid result, a borderline result is clinically interpretable and should be followed by retesting as a substantial proportion of individuals may test positive upon retesting. In a study of US healthcare workers, 23% of subjects with a borderline test result retested as positive, suggesting the borderline category is useful in maintaining test sensitivity. According to the CDC's Updated Guidelines for Using Interferon Gamma Release Assays to Detect *Mycobacterium tuberculosis* Infection published in 2010, "incorporation of a borderline category for the [T-SPOT.*TB* test] as approved by FDA increases test accuracy by classifying results near the cut point (at which small variations might affect the interpretation) as neither positive or negative."

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Are borderline T-SPOT.*TB* test results the same as invalid results?

No, borderline results are clinically interpretable whereas invalid results cannot be interpreted due to the failure of the test's Positive and/or Nil (Negative) Control. In both cases, however, retesting by collecting another blood specimen is recommended.

37

What should I do with a borderline T-SPOT.*TB* test result?

Borderline results are clinically interpretable and should be followed. Retesting by collecting another specimen is recommended. In a study of US healthcare workers, 23% of subjects with a borderline test result retested as positive, suggesting the borderline category is useful in maintaining test sensitivity. Upon retesting, if the test result remains borderline, other diagnostic tests and/or epidemiologic information should be used to help determine the TB infection status of the patient.

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What should I do with an invalid T-SPOT.*TB* test result?

Invalid results are not clinically interpretable and may occur if the Positive and/or Nil (Negative) Control does not perform as expected. Retesting by collecting another specimen is recommended. Upon retesting, if the test result remains invalid, other diagnostic tests and/or epidemiologic information should be used to help determine the TB infection status of the patient. Invalid results are uncommon and may be related to factors such as inappropriate blood storage conditions, delay in specimen transport, patient specific conditions, or laboratory error.

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Why does the T-SPOT.*TB* test include a Positive Control with each patient specimen?

The Positive Control serves as an indicator of patient cell functionality. Patient cells are incubated with a non-specific stimulator, phytohemagglutinin, in the Positive Control specimen well. The Positive Control spot count is ≥ 20 in the vast majority of patient specimens. A failed Positive Control is uncommon but may be related to factors such as inappropriate blood storage conditions, delay in specimen transport, technical factors or patient specific conditions. A small proportion of patients may have T cells which show only a limited response to phytohemagglutinin.

40

Why does the T-SPOT.*TB* test include a Nil (Negative) Control with each patient specimen?

The Nil (Negative) Control is designed to control for non-specific T cell reactivity. Patient cells are incubated with sterile media in the Nil Control well. For the test to be considered valid, there must be ≤ 10 spots in the Nil Control well. A failed Nil Control is uncommon but may be related to technical factors or a patient specific condition.

T-SPOT.*TB* methodology

41

How do I prepare blood specimens for the T-SPOT.*TB* test?

Blood specimens are processed using standard laboratory techniques and equipment to separate the blood cell fractions. Detailed information on specimen preparation can be found in the T-SPOT.*TB* package insert.

42

How is the T-SPOT.*TB* test performed?

The T-SPOT.*TB* test is a simplified and validated ELISPOT (enzyme-linked immunospot) method, which has some similarities to a conventional ELISA (enzyme-linked immunosorbent assay) method. A whole blood specimen is collected from the patient and sent to the laboratory. The laboratory separates the peripheral blood mononuclear cells (PBMCs), washes and counts them. A standard number of PBMCs are added to four microtiter wells, where they are exposed to a Positive Control, Nil (Negative) Control and two *Mycobacterium tuberculosis* specific antigens (ESAT-6 and CFP10). After overnight incubation, a conjugated antibody is added. The plate is then washed and a soluble substrate is added. Finally, the plate is washed and the numbers of spots are enumerated to determine the patient's result.

43

Are all the materials required for the test provided in the kit?

The T-SPOT.*TB* test kit contains the core materials required to successfully run the assay. The kit is designed to provide 4 wells per patient, a Positive and Nil (Negative) Control well and 2 other wells for incubation with the 2 supplied TB antigens. A list of additional required materials can be found in the T-SPOT.*TB* package insert.

44

How many tests (patient specimens) can be processed with the T-SPOT.*TB* 8 kit?

Each kit processes 24 patient specimens. The T-SPOT.*TB* 8 kit contains twelve strips of eight wells. Each strip processes 2 patient specimens (4 wells each), providing the flexibility of processing between 2 and 24 patient specimens per batch, without wasting strips.

45

What is the T-Cell *Xtend* reagent and how is it used?

The T-Cell *Xtend* reagent contains a bispecific monoclonal antibody that cross-links granulocytes with red blood cells, creating a complex which is removed during centrifugation. Lab personnel add the T-Cell *Xtend* reagent to whole blood prior to density gradient separation of peripheral blood mononuclear cells (PBMCs). T cells separated from whole blood stored over 8 hours appear to show reduced responses to antigen stimulation due to activated granulocytes which, suppress *in vitro* interferon-gamma production. By removing activated granulocytes, the T-Cell *Xtend* reagent extends blood stability to 32 hours after specimen collection.

The T-Cell *Xtend* reagent was approved by the FDA in 2010. Data supporting the approval is contained within the T-SPOT.*TB* package insert.

46

How long can blood specimens be stored prior to processing with the T-Cell *Xtend* reagent?

Blood specimens should be processed within 32 hours of specimen collection. Please refer to the T-SPOT.*TB* package insert for detailed information regarding specimen stability.

47

Does the T-Cell *Xtend* reagent impact T-SPOT.*TB* test performance?

Internal validation protocols and peer-reviewed studies have evaluated T-SPOT.*TB* test performance with and without the use of the T-Cell *Xtend* reagent and found high concordance and sensitivity.

48

Is a standard curve required each time the T-SPOT.*TB* test is performed?

No. Standard curves are not applicable to enzyme-linked immunospot (ELISPOT)-based tests, such as the T-SPOT.*TB* test. ELISPOT-based tests directly capture cytokines, such as interferon-gamma, secreted by cells as they are being released, at the single-cell level. In contrast, enzyme-linked immunosorbent assays, or ELISA-based tests, determine the total concentration of cytokines, such as interferon-gamma in plasma, relative to a standard curve.

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Which buffer should be used for washing the 96-well plate for the T-SPOT.*TB* test?

Sterile phosphate buffered saline (PBS) should be used.

50

What is the purpose of the cell washing and counting steps in the T-SPOT.*TB* test?

The cell washing step enables removal of plasma and potentially interfering substances, such as endogenous interferon-gamma, tricyclic antidepressants and nonsteroidal anti-inflammatory drugs. After washing, the peripheral blood mononuclear cells (PBMCs) are counted to allow for an adjustment in cell concentration to correct for variations in patient PBMC counts and ensure a standard number of cells are used in the test. The washing and counting steps may be of particular importance in patients with weakened immune systems. Immunosuppressed and immunocompromised patients have a higher risk of progressing from latent TB infection to TB disease, and may be prescribed a potentially interfering substance or have a PBMC count outside of normal values.

51

Can finished assay plates be stored for future reading?

Yes. Once developed, the spots in completed test plates remain stable and do not need to be read immediately. Completed test plates may be archived for retrospective quality control or re-examination, for up to 12 months, as long as they are properly stored in a dry, dark, room temperature environment.

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Is there a dedicated T-SPOT.*TB* product support service for laboratories?

Oxford Immunotec provides comprehensive technical support to customers, which includes customized training, product evaluation support and troubleshooting assistance. Our Product Support Team, available at 1-833-682-6933, offers support to existing customers as well as new customers and laboratories interested in offering the T-SPOT.*TB* test.

Screening control programs

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What is the impact of TB testing on healthcare resources?

Studies have shown that the incorporation of the T-SPOT.*TB* test in control programs will reduce the overall cost of TB control. This is largely due to the elimination of significant indirect costs associated with use of a tuberculin skin test (TST) to both health care systems and patients.

TST requires the person being tested have at least two office visits. Up to one-third of individuals do not return to have their test read. This may result in wasted resources and potentially dangerous gaps when containing an outbreak. Indirect resource and labor costs associated with administering and reading a TST are relatively high. These are compounded by the fact that the solution used in a TST, once opened, has to be used within a short time period leading to potential waste of unused stock. False-negative TSTs and non-returners may convert to TB disease leading to morbidity and higher costs of treating disease (including onward transmission).

False-positive TST results, often due to cross-reactivity with BCG vaccine or environmental non-tuberculous mycobacteria can lead to unnecessary anti-TB treatment and associated toxicity testing and clinical follow-up.

Contact investigations

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In contact investigations, should a baseline T-SPOT.*TB* test be performed?

Testing too soon after exposure may lead to a false-negative test result, as the individual's cellular immune response to *Mycobacterium tuberculosis* may not yet be detectable. However, a baseline test may be useful in determining whether a person had a pre-existing infection prior to exposure. Such a baseline test should be performed as soon as possible following exposure since conversion typically occurs 2 – 8 weeks post exposure.

According to the CDC's Core Curriculum on Tuberculosis: What the Clinician Should Know, contacts of a person with TB disease who have a negative initial interferon-gamma release assay (IGRA) or TST within 8 weeks of exposure be retested 8 – 10 weeks after last exposure.

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