WHO operational handbook on tuberculosis

Module 3: Diagnosis

Tests for tuberculosis infection

World Health Organization

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Web Annex. Use of alternative interferon-gamma release assays for the diagnosis of TB infection

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Abbreviations and acronyms

AIDS	acquired immunodeficiency syndrome
BCG	bacille Calmette-Guérin (vaccine)
CFP-10	culture filtrate protein 10
CXR	chest radiography (X-ray)
ELISA	enzyme-linked immunosorbent assay
ELISPOT	enzyme-linked immunosorbent spot
ESAT-6	early secretory antigenic 6 kDa
HIV	human immunodeficiency virus
IGRA	interferon-gamma release assay
IT	information technology
LMIC	low- and middle-income countries
mRNA	messenger ribonucleic acid
NTP	national TB programme
PBMC	peripheral blood mononuclear cell
PLHIV	people living with HIV
PPD	purified protein derivative
PPDS	standard PPD
QA	quality assurance
QC	quality control
QFT	QIAGEN QuantiFERON®-TB
SOP	standard operating procedure
TB	tuberculosis
TBST	*Mycobacterium tuberculosis* antigen-based skin tests
TNF	tumour necrosis factor
TPT	tuberculosis preventive treatment
TST	tuberculin skin test
TWG	technical working group
WHO	World Health Organization

1. Introduction

1.1 Rationale for TB infection testing

Tuberculosis (TB) infection is a state of persistent immune response to *Mycobacterium tuberculosis* antigens with no evidence of clinically manifest TB disease *(1)*. People with TB infection have no signs or symptoms of TB disease, are not infectious, have normal or stable images on chest X-ray (CXR), and have negative microbiological tests, if performed *(1)*. It is estimated that about 25% of the world's population has been infected with *M. tuberculosis (2)*, of whom 5–10% will develop TB disease over their lifetime *(3)*. This risk is much higher in those with certain epidemiological factors (e.g. recent contact with bacteriologically confirmed pulmonary TB), social characteristics (overcrowded and poorly ventilated living conditions, low socioeconomic status, or malnutrition) and demographical characteristics (e.g. very young children), or clinical conditions that compromise the immune system (e.g. HIV infection, or immunosuppressive medications) *(3–6)*. TB preventive treatment (TPT) for people with these risk factors can provide important individual and public health benefits. However, implementing TPT raises various challenges in terms of programme prioritization, health care worker reluctance to treat asymptomatic persons, medication adherence, availability of appropriate drugs and formulations, costs, burden on health systems and individuals concerned, and access to free tests for TB infection. TPT can induce adverse drug reactions (although these are rarely serious) in people who are generally healthy. Hence, TPT is recommended only to groups at high risk of developing TB disease *(1)*, in whom the benefits of TPT clearly outweigh the risks. Box 1.1 lists current World Health Organization (WHO) recommendations relevant to TPT provision.

In people of any given age (especially children and adolescents), health condition or epidemiological risk factor, the risk of TB disease is higher in those who have a positive TB infection test than in those with the same risk factors but a negative TB infection test *(4–6)*. Furthermore, TB infection testing is useful because individuals who test positive are more likely to benefit from TPT than those who test negative *(7, 8)*. Therefore, TPT will be of greatest individual and public health benefit if it is directed by an assessment of risk alongside the results of TB infection testing. Hence, extension of TPT to address global requirements creates a need to establish large-scale TB infection testing capacity.

Box 1.1. WHO TPT recommendations relevant to TB infection testing *(1)*

Adults and adolescents living with HIV who are unlikely to have active TB should receive TB preventive treatment as part of a comprehensive package of HIV care. Treatment should also be given to those on antiretroviral treatment, to pregnant women and to those who have previously been treated for TB, irrespective of the degree of immunosuppression and even if LTBI testing is unavailable.[a]
(*Strong recommendation, high certainty of evidence*)

Children aged <5 years who are household contacts of people with bacteriologically confirmed pulmonary TB and who are found not to have active TB on an appropriate clinical evaluation or according to national guidelines should be given TB preventive treatment even if LTBI testing is unavailable.
(*Strong recommendation, high certainty of evidence*)

Children aged ≥5 years, adolescents and adults who are household contacts of people with bacteriologically confirmed pulmonary TB who are found not to have active TB by an appropriate clinical evaluation or according to national guidelines may be given TB preventive treatment.[b]
(*Strong recommendation, low certainty of evidence*)

People who are initiating anti-TNF treatment, or receiving dialysis, or preparing for an organ or haematological transplant, or who have silicosis should be systematically tested and treated for LTBI.
(*Strong recommendation, low to very low certainty of evidence*)

Systematic LTBI testing and treatment may be considered for prisoners, health workers, immigrants from countries with a high TB burden, homeless people and people who use drugs.
(*Conditional recommendation, low to very low certainty in the estimates of effect*)

Systematic LTBI testing and treatment is not recommended for people with diabetes, people who engage in the harmful use of alcohol, tobacco smokers and underweight people unless they also belong to other risk groups included in the above recommendations.
(*Conditional recommendation, very low certainty in the estimates of effect*)

HIV: human immunodeficiency virus: IGRA: interferon-gamma release assay; LTBI: latent tuberculosis infection TB: tuberculosis; TBST: *Mycobacterium tuberculosis* antigen-based skin test; TNF: tumour necrosis factor; TPT: tuberculosis preventive treatment; WHO: World Health Organization.

[a] Consideration: TB infection testing is not a requirement for initiating TPT in PLHIV. PLHIV who have a positive test for TB infection benefit more from TPT than those who have a negative TB infection test *(7, 8)*; TB infection testing can be used, where feasible, to identify such individuals.

[b] Consideration: In this group the confirmation of TB infection using either IGRA, or TST or TBST, would be desirable. TB infection treatment may be justifiable without a TB infection test based on an assessment of the individual's risk of exposure and for the development of active TB in a given setting.

1.2 TB infection tests that are currently recommended by WHO

In 1908, Mantoux performed the first intradermal test for TB infection using killed *M. tuberculosis (9)*. The basic principles of this test have not changed in more than 100 years, making the tuberculin skin test (TST) one of the oldest tests still in clinical use. The skin test that is in widespread use today comprises the intradermal injection of a purified protein derivative (PPD) from heat-killed cultures of *M. tuberculosis*, with measurement of the resultant induration 48–72 hours later. Although standardized, this PPD material comprises a heterogeneous mixture of TB antigens, which results in significant cross-reactivity with other *M. tuberculosis* complex strains – notably bacille Calmette-Guérin (BCG) and nontuberculous mycobacteria *(10–12)*, reducing specificity.

Over the past 20 years, more specific tests for TB infection have been developed; these primarily use the early secretory antigenic 6 kDa (ESAT-6) protein and culture filtrate protein 10 (CFP-10), which are antigens that are found in *M. tuberculosis* but are not present in BCG or in most species of nontuberculous mycobacteria *(13)*. Testing for these two antigens has been performed using interferon-gamma release assays (IGRAs) on a blood sample. These tests measure interferon-gamma production from whole blood or from circulating lymphocytes after in vitro overnight incubation in the presence of ESAT-6 and CFP-10. More recently, intradermal skin tests targeting the same two antigens (*M. tuberculosis* antigen-based skin tests, TBSTs) have become commercially available. Since 2022, WHO has recommended the use of the new TBSTs as a way to test for TB infection, alongside older recommendations on IGRAs and TST (Box 1.2).

Box 1.2. Summary of WHO recommendations on TB infection tests *(14)*

Mycobacterium tuberculosis antigen-based skin tests (TBSTs) may be used to test for TB infection.
(Conditional recommendation for the intervention, very low certainty of the evidence)

Either a tuberculin skin test (TST) or interferon-gamma release assay (IGRA) can be used to test for LTBI.
(Strong recommendation, very low certainty of the evidence)

IGRAs (and the TST) should not be used in low- and middle-income countries for the diagnosis of pulmonary or extrapulmonary TB, nor for the diagnostic work-up of adults (including HIV-positive individuals) suspected of active TB in these settings.
(Strong recommendation)

HIV: human immunodeficiency virus; IGRA: interferon-gamma release assay; LTBI: latent tuberculosis infection; TB: tuberculosis; TBST: *Mycobacterium tuberculosis* antigen-based skin test; TST: tuberculin skin test; WHO: World Health Organization.

1.3 The TB infection cascade of care

Identifying, testing, evaluating and treating people with TB infection is a multistep process that has been termed the TB infection cascade of care *(15)*. The cascade is described in detail in Chapter 4. In brief, it comprises several steps that may involve health care personnel in different locations. Losses can occur at every step of the TB infection cascade. A systematic review showed that these losses resulted in less than 20% of those eligible for TPT completing their course of medication *(15)*. Importantly, under field conditions, 70–80% of the losses occurred before the initiation of TPT. Testing for TB infection can also be a barrier to the uptake of TPT, for various reasons including the unavailability of test materials, inadequate personnel to administer and interpret the tests, or the need for multiple visits. In summary, although testing for TB infection is not mandatory for the highest risk groups, it can provide important benefits to individuals and programmes, to target action to those more likely to benefit from it, avoid unnecessary treatment and enhance confidence of both provider and the recipient. However, important practical and programmatic challenges remain that limit access to TB infection testing. The impact of these challenges on the cascade of care must be considered at local level, and steps taken to resolve identified challenges.

1.4 About this operational handbook

This handbook reviews who should be tested for TB infection, and why and how they should be tested under programmatic settings. It provides information about the specific TB infection tests, from the original TST to the recently approved TBSTs, and the blood-based IGRAs, which are categorized as tests that measure interferon-gamma production using methods based on enzyme-linked immunosorbent assay (ELISA) or enzyme-linked immunosorbent spot (ELISPOT). Finally, programmatic aspects of the implementation and scale-up of TB infection testing are explained.

1.5 Target audience

This handbook is intended for use by TB and HIV programmes, and other experts involved in planning and implementing new or expanded programmes for TB infection testing. It is also intended for people involved in training, monitoring and supervision, and providers performing these tests. The annexes provide additional details, and educational materials about TB infection testing for providers and patients.

2. Testing for TB infection and a model algorithm

2.1 Who should be tested for TB infection?

The decision to test an individual for TB infection implies an intention to offer TPT. TB infection testing should therefore be reserved for populations in whom the risk of developing TB disease is high and who will benefit the most from TPT. Decisions to start TPT should always consider risk of adverse drug events, in addition to TB symptoms and the TB infection test result. Box 2.1 summarizes the groups WHO recommends should receive TPT, with testing not mandatory for groups 1–3. More detail can be found in Module 1 of the WHO operational handbook on TB *(1)*.

Box 2.1. Who should be considered for TPT based on WHO recommendations?

1. PLHIV. Adults, adolescents and children aged 12 months and older living with HIV who are unlikely to have TB disease should receive TPT as part of a comprehensive package of HIV care (regardless of ART, prior TB treatment or pregnancy).

2. Infants aged below 12 months living with HIV who are in contact with a person with pulmonary TB and who are unlikely to have TB disease.

3. Household contacts with bacteriologically confirmed pulmonary TB (regardless of age or HIV status).

4. People initiating anti-TNF treatment, or receiving dialysis, or preparing for an organ or haematological transplant, or who have silicosis.

5. Prisoners, health care personnel, immigrants from countries with a high TB burden, homeless people and people who use illicit drugs may be considered for TB infection testing and TPT.

ART: antiretroviral therapy; HIV: human immunodeficiency virus; PLHIV: people living with HIV; TB: tuberculosis; TNF: tumour necrosis factor; TPT: tuberculosis preventive treatment; WHO: World Health Organization.

2.2 Risk of disease in those with positive tests for TB infection

Numerous studies have identified risk factors for TB disease in people with positive or negative tests for TB infection; these risk factors may be epidemiological (e.g. contacts), demographical (e.g. age) or clinical (e.g. HIV infection). These studies have recently been summarized, and pooled estimates of absolute and relative risks provided, in an aggregate data meta-analysis *(6)*, and two large-scale individual patient data meta-analyses *(4, 5)*. The risk of TB disease varies widely among those with different epidemiological or clinical risk factors. In large-scale cohort studies of untreated people with positive TB infection tests but no other identifiable risk factors, the annual incidence of TB disease ranged from 0.3 to 1.0 per 100 000 *(6, 16, 17)*. By contrast, the cumulative risk of TB disease in those aged 2–5 years ranged from 50 to 250 per 100 000 in close contacts, particularly young children, or in people living with HIV (PLHIV) (i.e. more than 100 times higher).

Other reviews have summarized risks relative to incidence in general population samples (i.e. populations with positive TB infection tests but no other risk factors). The magnitude of the difference in risk varies; for example, it is 25 times greater in PLHIV without treatment and in recipients of dialysis, solid organ transplants or tumour necrosis factor (TNF)-alpha inhibitors, whereas it is two to five times greater in people with diabetes mellitus or alcohol use disorders, or who are underweight living in insanitary conditions and of low socioeconomic status *(18)*. This underscores the recommendations to consider TPT only for those at high risk, as listed in Box 2.1.

2.3 What are the advantages and disadvantages of testing for TB infection?

Testing for TB infection will be beneficial from an individual and a programmatic perspective if it identifies people who will benefit most from TPT. From a programmatic perspective, investments into capacity for TB infection testing will be justified if this results in greater efficacy and efficiency in the use of resources to provide TPT, increased acceptance and enhanced coverage. This will include not only the cost of drugs but also the human resources for medical evaluation, TPT initiation and follow-up. Testing will also save unnecessary expenditure on medication or adverse events to those receiving unnecessary treatment. Therefore, testing for TB infection before TPT is a valuable way to increase the TPT benefit–risk ratio.

The use of TPT has been shown to reduce the risk of developing TB disease among PLHIV, particularly in those who were TST positive. The updated systematic review undertaken during the development of the WHO guidelines on programmatic management of TPT in 2018 clearly demonstrated benefits of systematic testing and treatment of TB infection among PLHIV in terms of prevalence of TB infection, risk of progression to TB disease and incidence of TB disease when compared with the general population.

Household contactswere found to be at a substantially higher risk for progression to TB disease than the general population. The highest risk progression to active disease was among contacts who were aged below 5 years; hence, a strong recommendation to start TPT irrespective of

availability of test for TB infection was issued. In addition, TPT was conditionally recommended for household contacts in other age groups following assessment of harms versus benefits. Among household contacts aged above 5 years, TB infection testing before TPT initiation may be desirable, although treatment was considered justifiable even without testing *(19)*.

Among other risk groups, the evidence of benefits from systematic TB infection testing and TPT varied. The benefits clearly exceeded the risks among people starting anti-TNF treatment, receiving dialysis, preparing for an organ or haematological transplant, or having silicosis. In other risk groups, the risk versus benefit was less clear. Therefore, prioritization of target groups for systematic testing and TPT based on individual risk and the local and national context was considered to be acceptable to people with TB infection and to key stakeholders, including clinicians, nurses and programme managers.

In summary, TPT will provide the greatest individual health benefits if given to individuals with clinical or epidemiological characteristics that increase the risk of TB disease. Among those with a specific risk factor, the benefit will be maximized by prioritizing TPT for people with a positive TB infection test. However, testing is not a prerequisite in contacts of TB patients who are aged below 5 years and PLHIV. Hence, TB infection testing is associated with significant advantages for individuals.

The most important disadvantage of TB infection testing is the potential for significant delays between initial identification of someone at risk of developing TB disease and TPT initiation. In contacts, particularly young children *(19)* and PLHIV, TB disease can develop rapidly after exposure and TB infection. In all contacts, the highest risk period for progression to TB disease is within the first 6 months after exposure *(4, 5)*. Hence, prompt initiation of TPT is crucial to prevent TB disease. TB infection testing may contribute to substantial delays, either owing to lack of trained personnel to administer the test or read the skin test result, or to delays in laboratory processing and communication of IGRA test results. Since the results of IGRA testing should be available within 24–36 hours (although there may be additional delays due to sample transport and batch testing) and within 72 hours for TST or TBST, TB infection testing should not delay the initiation of TPT by more than 3 days after initial identification.

The second potential challenge with testing is the greater burden on patients, including discomfort, fear of injections or blood collection, and the need for more visits before starting TPT with associated potential patient costs, time, delays and resulting losses from the cascade of care. However, effective organization of health services can minimize cascade of care losses related to testing *(15, 20)*, both in high-income countries and in low- and middle-income countries (LMIC).

False negative and indeterminate TB infection tests are a third potential challenge *(21)*. Such test outcomes are more frequent among immunocompromised individuals. However, the high relative risks of developing TB disease in people with positive TB infection tests compared with those with negative tests suggests that false negative results are not major determinants of patient-important outcomes. Additionally, some people at risk (e.g. older contacts) may test negative but become infected later, or show infection shortly after; in such cases, not giving TPT would be a missed opportunity to protect people.

2.4 When is TB infection testing not advised?

This section sets out the various situations in which TB infection testing is not advised.

2.4.1 Prior positive TB infection tests

If a prior positive TB infection test or TB treatment is documented, then repeat TB infection testing will not be useful and should not be done. Depending on the circumstances, the individual may be referred for further medical evaluation. However, if a prior positive result is self-reported and not documented, it is recommended to repeat the test, because studies have documented highly inaccurate self-reporting of prior skin test results *(22)*.

2.4.2 Concomitant or recent vaccines or viral illnesses

TB infection testing may result in false negatives in individuals with certain viral illnesses (e.g. measles) or live virus vaccination (e.g. measles or mumps) within the preceding 30 days *(23)*. This has been described with TST, but a similar effect with all TB infection tests is biologically plausible. Hence, it may be appropriate to delay the TB infection test for 30 days after infection or vaccination. Alternatively, a negative TB infection test may be repeated after 30 days.

A common question in recent years has been the impact of coronavirus disease (COVID-19) infection or vaccination on TB infection testing. To date, no studies of TST or IGRA results after COVID-19 vaccination have been published. Given what is known about the immunological response to COVID-19 mRNA vaccination, such vaccination would not be expected to change TST or IGRA results *(24)*. However, given that test results could (at least theoretically) be modified by immunization, it may be prudent to test before the vaccine or postpone testing for a few weeks after the vaccine where possible *(25)*.

2.4.3 Clinical work-up of adults to diagnose TB disease or monitoring of the response to treatment

TB infection tests should not be used for the diagnosis of pulmonary or extrapulmonary TB, nor should they be used for the diagnostic work-up of adults (including PLHIV) with presumed TB disease. TB infection tests should not be used for screening or to monitor the response to treatment for TB disease or TB infection.

2.4.4 History of TST or TBST allergic reactions (but IGRAs may be used)

Skin testing is not advisable in people with a history of allergic reaction to TST or TBST. Allergic reactions to TST (PPD or equivalent), such as a generalized rash that occurs within the first 24 hours, are seen in less than 1% of recipients *(26)*. If this has been well documented in the past, then it is wise to avoid repeating the test with the same tuberculin material. Currently, it is unclear whether use of an alternative tuberculin material would be safe. Anaphylaxis in response to tuberculin skin testing is extremely rare (1 per million) *(27)*; however, if there is well-documented anaphylaxis in response to TST, then TB infection skin testing should not be performed, even with TBST, until further safety information is available.

Loss of consciousness after TST administration due to a vasovagal reaction (simple fainting) is far more common than anaphylaxis.

2.4.5 Challenges with blood collection in young children when using IGRAs (but TBST may be used)

IGRA TB infection tests should not be used in children aged 6 months to 2 years, because of insufficient data and the challenges of phlebotomy in this age group. TST or TBST may therefore be preferred. Testing household contacts below 5 years of age is not a prerequisite for providing TPT.

2.5 An integrated and person-centred model algorithm

To avoid barriers or delays created by TB infection testing, and to minimize associated losses in the TB infection care cascade, an integrated person-centred care model is preferred for TB infection diagnosis and treatment. Here, "integrated" means carrying out screening for TB disease and testing for TB infection in parallel, and "person-centred care" means coordination of multiple care activities during the same visits. This care model could shorten delays to appropriate treatment initiation, minimize time and cost burden on patients and their families, and promote retention in the TB infection cascade of care. Family-centred care is an extension of person-centred care, with care for the entire family coordinated to minimize time and cost burden on the entire family. This approach is particularly useful for contact investigation in households, is conducive to greater yield and retention on treatment, and is more cost-effective *(8)*.

In many settings, access to TB infection testing is currently suboptimal. Thus, making testing obligatory before starting TPT would create a barrier for many individuals who would benefit from such treatment and hence create a barrier for global expansion of TPT. To ensure TPT for individuals at particularly high risk of TB disease (e.g. child contacts aged below 5 years and PLHIV), current WHO guidance strongly recommends TPT even without TB infection testing.

An integrated algorithm for TPT among contacts (aged <5 years), PLHIV and other risk groups has been released by WHO as Module 1 of the guidelines *(1)*. For household contacts who are aged 5 years and older, and who are not HIV-positive, TB infection testing is advised as part of their care. Given that contacts are also at risk of TB disease, an integration of TB infection testing with TB screening would be an important step to enhance implementation.

In the **first visit**, as soon as someone at high risk of TB is identified, that person should undergo TB screening and at the same time should be tested for TB infection (Fig. 2.1). TB screening could be performed, for example, by assessing TB symptoms or using more sensitive WHO-recommended tools such as CXR, with or without computer-aided detection, molecular WHO-recommended rapid diagnostics or C-reactive protein (for PLHIV) *(28)*. Sequential screening and diagnostic testing (first for TB disease, then for TB infection) may incur substantial delays or losses in TB infection testing, particularly if these tasks are performed by different health care personnel or in different locations. Hence, during the first health care encounter, it is preferable to combine screening for TB disease with testing for TB infection. People with symptoms that are suggestive of TB disease should undergo further evaluation as soon as possible, preferably **on the same visit** *(29)*.

The **second visit** may be optimized for reading the TB skin test or obtaining the IGRA result; reviewing the results of microbiological tests for TB disease if the person had a positive TB screen and submitted samples for TB testing; undertaking clinical evaluation to rule out TB or before starting TB treatment; and starting TPT or TB treatment. This second visit should occur 48–72 hours after the test. If the TB infection test is negative, the individual can be discharged although aa repeat TB infection test may be required if an initial test is negative – particularly in those with very recent exposure or concomitant viral infection. When the TB infection test is positive in contacts who were asymptomatic initially, or who had symptoms but in whom TB disease was excluded, should be re-evaluated for immediate initiation of TPT (Fig. 2.1). Although TB infection tests may be positive in the presence of TB disease, their low accuracy means that these tests are not recommended for screening or as part of the diagnostic work-up of presumptive TB and this should be emphasized during the training of health care workers.

It is proposed to conduct TB infection testing early in the assessment of people at risk of TB (i.e. during the first visit) which helps to reduce delays in starting the appropriate treatment, and the test result may be helpful when deciding the best course of action in most of the people tested. If the person had no symptoms at screening, evaluation to exclude TB disease may still be warranted and CXR increases the sensitivity in such situations. Ideally, all these activities should be done on the same day, so that TPT can be prescribed during the second visit as soon as test results are available. Because most people with TB symptoms are not expected to have TB disease, deferring TB infection testing to a later stage (e.g. after further work-up) is such individuals may result in significant losses.

Fig. 2.1. Algorithm for person-centred TB infection care; integrated infection and disease assessment where TB infection testing is available[a] and recommended

Visit 1

TB infection testing
TB screening
Investigate for TB disease if screened positive for TB

Visit 2

TB infection test result
TB disease results (from Visit 1)
Investigate for TB disease if TBI test positive (and not done in Visit 1)
Start TPT or TB disease treatment

[Diagram nodes:]
TB screening[b] *and* TBI test[c] initiated including CXR if available

Screened negative for TB

Screened positive for TB

Evaluate and test for TB disease[d] (see Module 3)

No TB disease[e]

TBI test negative

TBI test positive

Discharge (or repeat TBI test later[f])

Start TPT[g]

TB disease start treatment

CXR: chest X-ray; IGRA: interferon-gamma release assay; PLHIV: people living with human immunodeficiency virus; TB: tuberculosis; TBI: TB infection; TBST: *Mycobacterium tuberculosis* antigen-based skin test; TPT: TB preventive treatment; TST: tuberculin skin test; WHO: World Health Organization.

[a] Where testing is not available (or not required based on local circumstances), the algorithm in the WHO operational handbook *(30)* can be used.

[b] TB screening performed using four TB symptoms (cough, fever, night sweats and weight loss). The WHO-recommended screening tools with high accuracy are preferred; they include CXR (with or without computer-aided detection), WHO-recommended rapid diagnostics or C-reactive protein (for PLHIV) *(28)*.

[c] TB infection tests include TST, IGRAs and TBSTs.

[d] A thorough clinical assessment should be performed to exclude TB disease (including extrapulmonary TB) and should include CXR where available. A WHO-recommended rapid diagnostic test should be used for diagnosis of TB disease where available *(31)*.

[e] Individuals screened using only symptoms initially should be carefully assessed for TB disease (including for extrapulmonary TB); the screening should include CXR where available, to rule out TB disease before initiating TPT.

[f] In circumstances where a test may be suspected to be false negative (e.g. concurrent viral infection), repeat testing may be considered after 30 days.

[g] Details on eligibility criteria and regimen choices can be found in the relevant WHO guidelines *(1)*.

The programmatic decision to initiate TB infection testing implies a commitment to start TPT rapidly where indicated. Hence, the programme should ensure that the referral pathways and medical services are well organized before launching testing for TB infection, and that the mechanisms for clinical assessment, start of medication and treatment support are all available (Chapter 4). An advantage of skin testing for TB infection (using TST or TBST) is the capacity to administer the test and read the result at the point of care. The portable nature of the supplies and materials needed for the test mean that testing can be done at home or in remote communities. However, these advantages are lost if the subsequent evaluation to exclude TB disease and the provision of TPT are not equally accessible. Hence, a decision to expand access to testing for TB infection needs to be matched by efforts to expand access to the medical services needed for management of those with positive test results.

3. WHO-recommended tests for TB infection

3.1 TB infection skin test using tuberculin (TST)

The original tuberculin material used by Mantoux in his first studies of tuberculin reactions was a highly heterogeneous mix of substances from killed *M. tuberculosis (9)*. This so-called old tuberculin was replaced in 1941 by a standardized preparation of PPD from *M. tuberculosis*. A single standard lot of this material was produced by Florence Seibert, termed "PPDS" *(32)*; since then, all newly produced tuberculin material has been produced using the same methods and tested against this standard, measuring induration in sensitized guinea pigs. All commercially available tuberculin material, other than the "next generation" skin test described later, are manufactured to produce PPD material that is bioequivalent to this standard PPDS.

PPDS contains a mix of antigens, including some that are specific to *M. tuberculosis*, but also many that are found in nontuberculous mycobacteria and BCG. Hence, false positive reactions to PPDS have been described in people with nontuberculous mycobacterial disease or with sensitization to nontuberculous mycobacteria antigens *(12)*, and in people who have received BCG vaccination, particularly if they received BCG more than once or after infancy *(11)*.

Testing with PPDS is very safe. Although severe local reactions with blistering can be seen in 2–3% of people, these are true positive reactions that are self-limited and heal spontaneously. Allergic reactions, with generalized rash, occur in less than 1% of people *(26)*, and anaphylaxis occurs in only 1 per million people *(27)*. Based on decades of experience, TST with PPDS is considered safe in pregnant and lactating women *(33)*.

3.1.1 Interpretation of TST – criteria for a positive TST

The sensitivity of TST based on different size criteria of induration was established among people who had been treated for and had recovered from microbiologically confirmed TB *(34)*. Of these, 98% had a reaction of 5 mm or more to TST, 90% had a reaction of 10 mm or more but only 60% had a reaction of 15 mm or more. On the other hand, reactions to TST due to BCG or nontuberculous mycobacteria are usually smaller than TST reactions due to true infection with *M. tuberculosis (11, 12)*. Therefore, setting higher cut-off points for a positive TST will improve specificity, but will reduce sensitivity, especially if a cut-off point of 15 mm is used *(35)*. Hence, most clinical guidelines suggest using different size criteria for different populations, epidemiological situations and comorbidities (Table 3.1). If risk of TB disease is high, then sensitivity should be maximized; however, if risk of TB disease is low (or risk of adverse events from TPT is high), then cut-off points should be adjusted upwards to maximize specificity. In

groups for which systematic TB infection testing and TPT are not recommended, an incidental finding of positive TST beyond the thresholds in Table 3.1 should prompt further queries about other risk factors that may warrant TPT (e.g. contact with an infectious TB patient). Otherwise, these individuals may be advised to return to health care immediately if symptoms associated with TB emerge.

Table 3.1. Criteria for size of TST induration by population (based on studies using PPDS or bioequivalent) *(36)*

Criteria (diameter of induration)	Epidemiological and clinical conditions
5 mm	PLHIV
	Severely malnourished children
	A recent contact of a person with pulmonary TB disease
	Renal failure (dialysis)
	Silicosis
	Patients undergoing preparation for a solid organ transplant and other immunosuppressed individuals who are on cytotoxic agents such as cyclophosphamide or methotrexate
	People who are immunosuppressed for other reasons (e.g. taking the equivalent of >15 mg/day of prednisone for 1 month or longer, or taking TNF-α antagonists)
10 mm	Recent immigrants (within 5 years) from high prevalence countries
	People with alcohol use disorder and injection drug users
	Residents and employees of high-risk congregate settings (e.g. prisons, nursing homes, hospitals and health facilities, and homeless shelters)
	People with clinical conditions that place them at high risk (e.g. diabetes, prolonged corticosteroid therapy, leukaemia, endstage renal disease, chronic malabsorption syndromes and low body weight)
	Malnutrition
	Health care personnel
	Children aged below 5 years of age, or children and adolescents exposed to adults in high-risk categories
15 mm	Low-risk,[a] healthy individuals with no specific epidemiological or clinical risk factors

PLHIV: people living with human immunodeficiency virus; PPDS: standard purified protein derivative; TB: tuberculosis; TNF: tumour necrosis factor; TST: tuberculin skin test; WHO: World Health Organization.

[a] Current WHO guidelines do not recommend testing of low-risk individuals. An incidental finding would require further evaluation for other risks and close follow-up.

3.2 TB infection skin tests using *M. tuberculosis-specific antigens*

3.2.1 Cy-Tb

Cy-Tb (formerly known as the C-Tb test, Serum Institute of India) contains a 1:1 ratio of two recombinant proteins of ESAT-6 and CFP-10 was previously produced by genetically modified *Lactobacillus lactis,* by Statens Serum Institute (Denmark). One single test dose of 0.1 mL contains 0.05 µg of rdESAT-6 and 0.05 µg of rCFP-10.

Fig. 3.1. Vial of Cy-Tb

The manufacturer recommends administration using the Mantoux (i.e. intradermal) method. If there is a resultant reaction, the transverse diameter of induration only should be measured and recorded in millimetres after 48–72 hours *(37)*.

Safety

The safety of Cy-Tb has been assessed in seven studies with a total of 2924 participants *(38)*. No serious adverse reactions occurred in these seven studies. In four studies, all participants simultaneously received both Cy-Tb and Rt-23 (bioequivalent to PPDS *(39)*); in these studies, fever occurred in 32 of 1235 people (2.7%). However, it is difficult to be certain that the fever was due to Cy-Tb, given that all these people received both tests. Local injection site reactions are common; in five studies, participants received both Cy-Tb and Rt-23 simultaneously on opposite arms. Test administration was random and blinded, and assessment of local reactions was also blinded. In these five studies, the relative risk of local reactions was 1.05 (95% confidence interval (CI): 0.7 to 1.9) indicating no significant difference in local site reactions with Cy-Tb compared with traditional tuberculin skin tests. Over 95% of these local reactions were judged mild to moderate.

Pregnancy and lactation

To date, fewer than 300 pregnant women have received Cy-Tb, with no evidence of related adverse fetal outcomes *(37)*. Similarly, there is limited information on safety in lactating women, but no adverse effects on breastfeeding infants have been reported (nor are they expected, given the limited systemic absorption of the intradermal test material).

Contraindications

The only contraindication is allergy to products from *Lactobacillus lactis (37)*.

3.2.2 Diaskintest

Diaskintest (Generium, Russian Federation) is a recombinant protein produced by genetically modified culture of *Escherichia coli* BL21 (DE3)/pCFP-ESAT, diluted with sterile isotonic phosphate buffer solution, with a preservative (phenol). One dose (0.1 mL) of the product contains 0.2 µg of CFP-10–ESAT-6 recombinant protein, and excipients – disodium phosphate dihydrate, sodium chloride, potassium dihydrogen phosphate, polysorbate 80, phenol and water for injection *(40)*.

Fig. 3.2. Diaskintest package and vial

Source: Reproduced with permission of Generium, ©2021. All rights reserved.

The manufacturer recommends administration intradermally and reading of the reaction at the injection site 48–72 hours after injection. The presence of redness and induration should be noted, although the presence and size of induration is critical to interpretation. The presence of blistering, necrosis (skin breakdown) or lymphadenitis is rarely recorded and if seen is interpreted as a "hyperergic" reaction.

Safety

There is limited information on the safety of Diaskintest. Information from the manufacturer indicated that in 2019–2021 a total of 55 774 995 Diaskintest tests were done, in which 27 serious adverse effects and 30 non-serious adverse effects were reported. Unfortunately, there

is no further detail on the serious reactions. Published safety studies were recently reviewed systematically *(38)*. In one study of 53 patients with TB disease, six developed severe local reactions with Diaskintest compared with two people developing reactions with TST. In the second study of 25 health care personnel, one participant had a severe local reaction to Diaskintest compared with none with TST. Fever was documented in 32 of 1201 (2.7%) recipients of Diaskintest and other systemic reactions in 1 of 527 recipients (0.2%) *(38)*.

Pregnancy and lactation

The effect of the product on women during pregnancy and lactation has not been studied. Its effects in the developing fetus and reproductive function are unknown. Data on transfer of the product into breast milk during lactation are absent *(40)*.

Contraindications

Contraindications to Diaskintest are:

- acute and chronic (in the exacerbation period) infections, except for TB (possible or presumptive);
- somatic and other diseases during exacerbation;
- common skin diseases;
- allergic conditions;
- epilepsy; and
- hypersensitivity to the active substance or to any of the excipients in the product *(40)*.

3.2.3 C-TST

The active ingredient in C-TST (formerly known as the ESAT6-CFP10 test, Anhui Zhifei Longcom, China) is an ESAT-6–CFP-10 fusion recombinant protein expressed in genetically modified *E. coli*. Each test dose of 0.1 mL contains 5 U of recombinant *M. tuberculosis* fusion protein, and auxiliary ingredients – disodium hydrogen phosphate, potassium dihydrogen phosphate, sodium chloride, phenol and polysorbate 80 *(41)*.

Fig. 3.3. C-TST vials
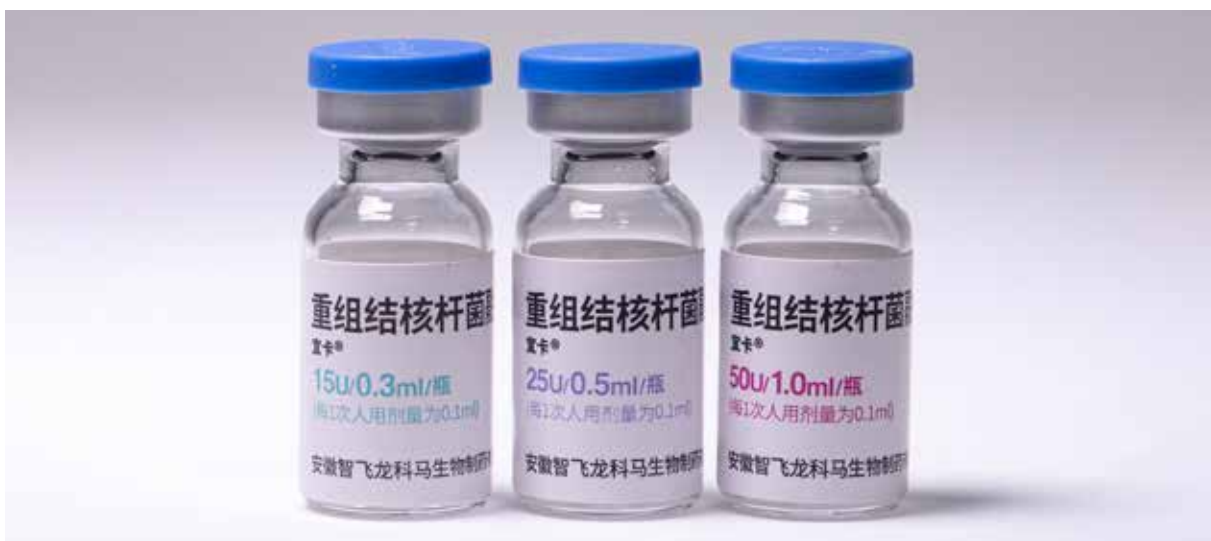
Source: Reproduced with permission of Anhui Zhifei Longcom, ©2021. All rights reserved.

The manufacturer recommends administration using the Mantoux method – intradermally on the inner aspect of the forearm below the level of the elbow (cubital fossa). The manufacturer then recommends reading of the reaction at the injection site 48–72 hours after injection. The transverse and longitudinal diameters of both redness and induration should be measured, and the average diameter of the redness or induration should both be recorded (calculated as the sum of the transverse and longitudinal diameters, divided by two). Whichever is the larger (either the redness or the induration) is selected as the result for clinical interpretation. The presence of blistering, necrosis (skin breakdown) or lymphadenitis is recorded and interpreted as a strong positive reaction *(41)*.

Safety

According to the manufacturer's product insert, seven clinical trials to evaluate safety were completed in China, involving a total of 3854 subjects. These included 861 patients with TB, 443 patients with other non-TB diseases, and 1984 healthy people of whom 361 had received BCG vaccination 11–13 weeks earlier, and 205 had received placebo vaccination at the same time. Most participants received C-TST and TST at the same time; all data related to systemic adverse reactions were collected from subjects who received TST (i.e. with PPDS) at the same time. These studies were included in a recent systematic review *(38)*. In two Phase 2B studies, adverse reactions were more frequent with C-TST than with standard TST (27.8% versus 16.5%, P<0.001). A single study assessed systemic reactions and noted that nine of 144 participants (6.3%) of C-TST developed systemic reactions; these included hypertension and vasculitis. In these studies, C-TST was administered at the same time as TST which makes attribution of systemic adverse events between tests difficult. Data from a phase III trial are awaited.

Pregnancy and lactation

Pregnancy and lactation are not mentioned in the product insert, and no data on C-TST in pregnancy and lactation are available.

Contraindications

Contraindications specific to C-TST, from the product insert, are:

- those aged under 6 months or above 65 years, relative contra-indication, as no data regarding safety or accuracy available in these age ranges; and
- patients with acute infectious diseases (e.g. measles, pertussis, influenza or pneumonia), acute meningitis with ocular involvements, acute otitis media and extensive skin diseases.

3.2.4 Interpretation of TBST – criteria for a positive TBST

To date, the criteria for a positive TBST have been established by the manufacturers. These criteria are based on clinical data in relatively small numbers of patients. In addition, all of the studies to date evaluating accuracy of these tests have used cross-sectional designs. Data from prospective observational or experimental studies may result in modification of these criteria in the future.

Table 3.2. Criteria for a positive TBST

Test	Criteria[a]
Diaskintest	Negative: absence of infiltration (induration) or hyperaemia or the presence of a "prick response" up to 2 mm
	Ambiguous: the presence of hyperaemia without infiltrate (induration)
	Positive: the presence of infiltrate (induration or papule) of any size
	Poorly expressed response:[b] infiltration size up to 5 mm
	Moderately expressed response[b]: infiltration size 5–9 mm
	Pronounced response:[b] infiltration size 10–14 mm
	Hyperergic response: infiltration size 15 mm or more, with vesiculonecrotic alterations or lymphangitis or lymphadenitis, regardless of the infiltration size
Cy-Tb	Induration ≥5 mm
C-TST	Average diameter (sum of transverse and longitudinal diameters, divided by 2) of redness or induration ≥5 mm
	Blister, necrosis (skin breakdown) or lymphadenitis are interpreted as strong positive reactions

TBST: *Mycobacterium tuberculosis* antigen-based skin test.

[a] The criteria are all taken from manufacturers product inserts *(37, 40, 41)*.

[b] All responses are considered positive according to the package insert,

3.3 Steps and processes for skin testing

The general methods for testing administration and reading, described below, are derived mainly from published studies using TST; that is, skin testing with tuberculin material bioequivalent to PPDS *(42)*. Safety of TST (using tuberculin material that is bioequivalent to PPDS) has been well established, based on millions of doses received. However, safety is much less established for the new TBST, because there is less experience in using TBST. Therefore, surveillance of adverse events in countries initiating testing with the new TBST should be implemented. Chapter 2 (above) discusses general indications and contraindications for TB infection testing. A detailed step by step instruction on skin testing is provided in Annex 1.

3.3.1 Materials and supplies needed

Skin test material (see above for details for each tuberculin and *M. tuberculosis*-specific antigen) should not be exposed to heat or light, and it should be refrigerated when not in use, but not frozen. Therefore, a cold chain needs to be maintained from production until the point of administration, and appropriate equipment (e.g. refrigerators, ice packs and coolers) must be provided. Tuberculin material should be drawn up in a syringe just before administration, and not stored over prolonged period after being drawn up in a syringe.

3.3.2 Personnel needed

Personnel involved in skin test administration should be health care workers who are trained and authorized to perform parenteral injection of medications or biological materials by the country's ministry of health. Reading of tests can be done by any trained health care professional.

3.3.3 Training and quality control

As with any diagnostic test, correct technique in administration and reading of the test is essential for accurate, reproducible and valid test results. Therefore, personnel require initial training, mentoring and ongoing supervision to maintain quality *(42)*. Training for reading of tuberculin reactions is ideally done at a large centre where large numbers of people receive TB infection skin tests regularly. In this way, in a relatively short amount of time, trainees can practise the reading of indurations of different sizes and consistency. On-site training is more complicated to organize when the supervisors are not present on site but make occasional monitoring visits.

Independent online resources are available to explain the optimal procedures for tuberculin skin test administration *(43)* and reading *(44)* (these can be adapted for use with other TBSTs).

Remote monitoring of TST administration and interpretation as an aid to staff training and quality control (QC) has been studied *(45)* (Box 3.1). In this study, this "mTST method" tested a procedure whereby individuals who administer and read TST are also trained to take photos (using a smartphone) of the administration and reading sites. These photos, combined with the initial readings by the health care worker, are sent to the supervisor and feedback is given (by email or phone). Online videos are available showing reviewers performing quality assurance (QA) for the TB infection skin test *(46)* and how to take mTST photos *(47)*.

Box 3.1. Remote monitoring of TST or TBST administration and results

Performing a TST or TBST correctly is relatively straightforward but it requires staff trained in administration of the test. Likewise, correct interpretation of the result requires staff to read the result with an additional health encounter. It becomes more likely that administration technique and TST or TBST reading can be done remotely as penetration of smartphones with high-definition cameras increases globally. One study using photos acquired via smartphone estimated the accuracy and reproducibility of measuring the size of swelling immediately following TST administration (TST injection bleb) and after 48–72 hours (TST induration) ("mTST method") *(45)*. Agreement between mTST reading of 64 digital photos of injection blebs and on-site human reading by an experienced TB nurse was high (κ 0.75–0.87), and the reproducibility of readings between six reviewers of photos was very high (κ: 0.86–0.96). Using the mTST method to assess TST induration in 72 photos, reviewers were able to detect no induration (<5 mm) with accuracy of 95% and induration of 15 mm or greater with accuracy of 92%; however, accuracy was only 20% for reactions of 5–9 mm and 77% for reactions of 10–14 mm. Thus, the "mTST method" seems to bear promise as a tool to assess TST or TBST administration, although it was less accurate for TST indurations of certain size. More validation will be needed before the tool can be relied upon to replace trained staff for in-person supervision and interpretation of TST or TBST results. Similar efforts to employ artificial intelligence to read images of skin test reactions taken using smartphone are currently underway and may become available in the near future to facilitate reading of test results.

3.3.4 Overview of test procedures and management

Administration of the TST or TBST (Mantoux method)

Before administration of the TST or TBST via the Mantoux method *(48)*, the person to be tested should be seated comfortably, with their arm supported. The administrator should take a few minutes to explain the procedure and ensure that the person understands and agrees to testing, and that they can and will return for reading within 48–72 hours. Confidentiality should be maintained, meaning that the testing is performed in a separate room, with only the individual being tested being present (accompanied by family members where appropriate). In cases where a child is administered the test, consent by a parent or guardian is required and the presence of the parent or guardian may be necessary.

The test is administered on the inner aspect of the forearm, about 10 cm below the elbow. This is conventional because the skin surface at that point is fairly flat, making subsequent measurement of induration easier. The spot selected should have no scars, rashes, burns or tattoos.

The tuberculin material is drawn up in the syringe not more than 20 minutes before injection, and the skin is cleaned and allowed to dry. The tuberculin syringe is laid flat on the skin, with

the bevel of the needle upward, as shown in an online video *(43)*. The material is injected slowly and should create a small bleb (at least 7 mm in diameter *(45)*), which will disappear in 15–20 minutes. The material must not be injected subcutaneously because this makes it more difficult to measure the result and can even lead to false negative results. It is not necessary to cover the area with a bandage or to mark the injection site in any way.

After test administration, the individual should be monitored for 10–15 minutes; that is, the individual should be asked to wait, ideally seated. One of the adverse reactions to note is vasovagal fainting, which is to be distinguished from anaphylaxis. Any dizziness or loss of consciousness should be carefully documented, so that, in the future, it is known whether the individual had a vasovagal reaction or anaphylaxis.

Upon discharge, patients should be instructed to keep the injection site clean. If there is significant swelling and itching, they should avoid scratching but can apply a cold compress. Patients can perform all normal activities including bathing or showering. The importance of a return visit within 48–72 hours for reading should be reinforced.

The person who administers the test should record the date, time and position on which forearm the test was administered; the name, manufacturer and lot number of the tuberculin material; and the date on which the vial was opened. Any adverse reactions should be noted.

Reading the resultant reaction

Patients should be instructed to return after 48–72 hours. If an individual fails to present after 48 hours, they can be contacted and reminded to come in the following day. This would allow reading to be performed within the maximum of 72 hours that is optimal for accuracy of all TB infection skin tests *(49)*.

Reading should be done with the patient seated comfortably and the arm supported, so that the skin is relaxed as much as possible. For all tests except C-TST, it is important to measure the induration but not the redness (for C-TST, see below). Induration is best measured using the ballpoint method *(50)*. The margins of the induration can be measured with a ruler or tape measure, but this can lead to a rounding error; hence, reactions are usually grouped at 5, 10 or 15 mm points. More precise measurements can be obtained using simple calipers used by machinists, mechanics or tailors.

The diameter of the induration should be recorded in millimetres, along with the interpretation (positive or negative) and referral for medical evaluation (if positive). Redness can be noted; any blistering should also be noted because this is synonymous with a positive test. If there is significant blistering or skin breakdown, the area should be cleaned and then covered with a dry dressing, simply to protect it from secondary infection. Management with topical steroids is ineffective *(51)*; cold compresses and prescription of oral analgesics (aspirin or acetaminophen/paracetamol) should be adequate.

Self-reading of test result

Studies have reported low accuracy of self-reading of the TB skin test result. One study noted that 50% of reactions that were read as positive by a trained health professional were interpreted as negative by the patient *(22)*.

Interpretation of TST or TBST

Criteria for interpretation of TST-PPDS have been established in numerous large-scale observational and intervention studies. The sensitivity of different criteria for size of induration was established in a multicentre international study conducted by WHO in the 1950s *(34)*. Numerous studies have demonstrated that the sensitivity of TST can be reduced because of technical problems with the test itself, or biological conditions that impair the cell-mediated immune response *(21, 42)*. These are summarized in Table 3.3. To date, there have been few studies of the new TBSTs to understand the impact of these problems on the sensitivity of these tests; in the absence of such data, it is assumed that sensitivity of the new TBSTs will be affected in the same way. Although technical issues can be identified and corrected, the biological causes of reduced sensitivity are more difficult to correct, so should be considered carefully when interpreting a negative TB infection skin test result.

Table 3.3. Potential causes of false negative TB infection skin tests[a]

Type of issue	Possible explanation
Problems with the product quality and testing technique	Tuberculin material expired or improperly stored (exceeded manufacturer-recommended maximum temperature specifications, or was frozen or exposed to direct light, e.g. sunlight)
	Tuberculin material drawn up in syringe more than 20 minutes before administration
	Poor administration technique (insufficient dose administered or injection too deep)
	Improper reading (e.g. rounding error or bias)
Biological factors	Active TB disease (untreated or newly diagnosed – especially if more advanced)
	HIV infection (especially if CD4 <100)
	Immunosuppressive medications:
	– transplant anti-rejection
	– TNF-alpha inhibitors (e.g. Infliximab or Etanercept)
	– corticosteroids (>20 mg day of prednisone or equivalent)
	– other immunosuppressive medications
	Malnutrition (severe)
	Recent viral infection – within 30 days (measles)
	Live virus (measles) vaccination within 30 days

HIV: human immunodeficiency virus; PPDS: standard purified protein derivative; TB: tuberculosis; TBST: *Mycobacterium tuberculosis* antigen-based skin test; TNF: tumour necrosis factor; TST: tuberculin skin test.

[a] All studies used TST (PPDS), but it is assumed that these problems would lead to similar reductions in sensitivity of TBSTs.

Many of the antigens found in *M. tuberculosis* are also present in nontuberculous mycobacteria (e.g. *M. avium*) and the BCG vaccine. These antigens that are not specific to *M. tuberculosis* commonly cause false positive reactions to TST. Since the likelihood of prior BCG vaccination or exposure to nontuberculous mycobacteria varies among different populations and clinical situations, the specificity of TST also varies, and is suboptimal in many populations *(42)*. Hence,

the criteria to define a positive TST varies according to clinical and epidemiological risk factors (see Table 3.1 above).

Specificity is of much less concern for the IGRAs, or for the new TBST, because these tests measure immune reactions to only two antigens (ESAT-6 and CFP-10) which are much more specific to *M. tuberculosis (13)*. These antigens are present in some nontuberculous mycobacteria such as *M. kansasii*, *M. szulgai* and *M. marinum (13)*, but these are uncommon. Hence, manufacturers of these tests suggest a single cut-off point to define a positive test that indicates "*M. tuberculosis* infection likely".

Referral for follow-up

If the TB infection test is negative the patient can usually be discharged (if asymptomatic), with counselling about potential future TB exposures and risk of infection. In some settings, although not a WHO recommendation, a second TB infection test is done 8 weeks after the end of exposure to identify delayed infections and late TB infection test conversions, particularly in the evaluation of people with significant exposure, such as household contacts of a contagious TB case.

If the TB infection test is positive the patient should be promptly referred for medical evaluation and consideration for TPT initiation. Ideally, medical evaluation, including CXR when available, should be performed on the same day as the reading of the test. This "one stop shop" approach will minimize delays in initiation of TPT, reduce losses in the TB infection cascade of care, and reduce patient costs and time lost from travel and waiting during multiple visits to a clinical facility.

Future testing

If the individual has a negative test, then they may be a candidate for future testing (if required as per national policy). If the individual has a positive test, they should not have a skin test in the future. Although skin tests can revert from positive to negative, the clinical interpretation of individuals who had a prior positive test and now have a negative or a more strongly positive test is unclear *(38)*.

3.4 Interferon-gamma release assays

IGRAs are in vitro blood tests that measure interferon-gamma released by circulating lymphocytes in whole blood during overnight incubation with exposure to *M. tuberculosis*-specific antigens (ELISA based) or the number of T-lymphocytes producing interferon-gamma (ELISPOT based). In 2011, WHO issued recommendations on the use of IGRAs for the diagnosis of TB infection, including the blood-based QIAGEN QuantiFERON-Gold (QFT-G), QuantiFERON-TB Gold In-Tube (QFT-GIT) and Oxford Immunotec T-SPOT.TB (T-Spot) assays. The QFT-G and QFT-GIT were discontinued by the manufacturer. More recently, QIAGEN released an updated test, QuantiFERON-TB Gold Plus (QFT-Plus), which superseded the QFT-G and QFT-GIT; also alternative blood-based IGRAs from other manufacturers have been marketed. To evaluate these technologies and determine whether one or more of them could be included under the existing WHO recommendations for IGRA testing, WHO convened a Technical Advisory Group on TB Diagnostics and Laboratory Strengthening. The group met virtually on

27–29 October 2021 and a subsequent WHO policy statement on the use of alternative IGRAs was issued subsequently (Web Annex).

The two new ELISA-based IGRA assays recommended by WHO for TB infection diagnosis are QFT-Plus and WANTAI TB-IGRA, while T-SPOT®.TB continues to be only ELISPOT-based IGRA that is recommended *(8)*. In contrast with TST, all currently WHO-recommended IGRAs require a well-equipped laboratory and trained laboratory technicians for testing. Because the commercially available currently recommended IGRAs are based on the lymphocyte response to *M. tuberculosis*-specific antigens (ESAT-6 and CFP-10), results are not affected by prior BCG vaccination, making these tests more specific than TST. The genes that produce these antigens are present in the following nontuberculosis mycobacteria: *M. kansasii, M. szulgai* and *M. marinum*. Challenges with phlebotomy in young children mean that these tests have limited applicability, with skin tests being the alternative. Details for each IGRA test are provided in Annex 2.

3.4.1 ELISA-based QFT-Plus

The QuantiFERON®-TB Gold Plus (QFT-Plus) assay is a test on whole blood; it uses peptides simulating ESAT-6 and CFP-10 proteins to stimulate lymphocytes in whole blood. This assay is produced by QIAGEN and is the fourth generation of QuantiFERON assays. QFT-Plus is a modification of the QFT Gold In-Tube test (QFT-GIT), and was developed to improve sensitivity in immunocompromised subjects and young children. QFT-Plus has four tubes, into each of which 1 mL of whole blood is drawn. One tube contains mitogen and acts as the positive control, one tube contains nothing and acts as the negative control. The third tube (TB1) is the same as the TB antigen tube of QFT-GIT and contains TB-specific antigens (ESAT-6 and CFP-10), while the fourth tube (TB2) has the same antigen mix as TB1, but the peptide lengths differ (short and long) to elicit cell-mediated immune responses from both CD4+ and CD8+ T-lymphocytes *(52)*.

Fig. 3.4. The QuantiFERON-TB Gold Plus (QFT-Plus) kits and tubes

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3.4.2 ELISA-based WANTAI TB-IGRA

WANTAI TB-IGRA was developed and is produced by Beijing Wantai Biological Pharmacy Enterprise Co Ltd, Beijing, China, and is similar to QFT-Plus. Test kits are supplied as three tubes: the first contains phytohemagglutinin acting as a positive control; the second does not contain any antigen and acts as a nil or negative control, and the third contains TB-specific antigens. In WANTAI TB-IGRA, the TB-specific antigens are a recombinant fusion protein of CFP-10 and ESAT-6 (expressed in genetically engineered bacteria) to elicit cell-mediated immune responses from CD4+ T-lymphocytes, whereas QFT-Plus uses polypeptide antigens of CFP-10 and ESAT-6 *(53)*.

Fig. 3.5. WANTAI TB-IGRA kits and components

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3.4.3 ELISPOT-based T-SPOT.TB

T-SPOT.TB is the only ELISPOT-based IGRA test that is currently recommended by WHO. This test is produced by Oxford Immunotec, United Kingdom of Great Britain and Northern Ireland (United Kingdom). The T-SPOT.TB test requires a normalization step in which peripheral blood mononuclear cells (PBMCs) are isolated, washed and counted to ensure the reproducibility of the test. The test has four wells into which isolated PBMCs are placed. One well contains nil (for a negative control), one phytohemagglutinin (for a positive control) and two the *M. tuberculosis*-specific proteins (ESAT-6 and CFP-10) *(54)*.

Fig. 3.6. T-SPOT.TB kits

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4. Programmatic considerations for implementing TB infection tests

4.1 General considerations: integrated and person-centred TB infection cascade of care

The decision by a ministry of health to initiate or expand TB infection testing requires preparedness of medical services. Many of the people who may be eligible for TPT will need to be able to access this important intervention. This means that referral pathways and health services must be planned or reorganized to ensure that all individuals among target populations are tested promptly and linked to appropriate treatment based on the test results. This is best accomplished by adopting a patient-centred approach that considers the individual's needs and perspectives. Programmes should streamline services to minimize delays and should coordinate delivery of health care services following the suggested simplified cascade of care (Fig. 4.1). Where possible, different activities in the TB infection cascade of care should be merged to reduce the number of visits needed and to integrate screening for TB disease and TB infection so that these two essential activities are performed together. This chapter reviews how NTPs should organize health services and plan resource allocation to ensure high-quality integrated and person-centred care for all steps of the TB infection cascade of care, to maximize retention in the TB infection cascade.

Fig. 4.1. Simplified four-step person-centred TB infection cascade of care

Step 1:
Identify target populations for TPT
Undertake TB screening and investigation of TB disease
Initiate TB infection testing

All health care personnel, in all programmes and all settings, identify target populations for TPT and screen for TB, assessing TB symptoms and preferably using more accurate tools (e.g. CXR) to detect TB disease; those with a positive TB screen are promptly investigated for TB disease. At the same visit, qualified personnel administer skin tests (TST or TBST) or draw samples for IGRA to detect TB infection.

Step 2:
Review TB infection test result
Assess for TB disease
Recommend and initiate TPT where appropriate

Trained health care personnel read and interpret the TB infection skin test result or obtain the IGRA result. If the test result is positive, they reconfirm that the earlier assessment satisfactorily excluded the likelihood of TB disease, then determine TPT eligibility through clinical evaluation, counsel the patient and initiate TPT, and complete the baseline recording during the same visit.

Step 3:
Follow up during TPT

Trained health care personnel oversee provision of TPT for early identification, recording and reporting of adverse events and follow the patient through TPT completion
Adopting shorter and safer TPT will improve this step.

Step 4:
Complete TPT

TPT completion will be facilitated by factors such as ongoing support during TPT and use of a shorter regimen. The final TPT outcome is recorded and reported.

CXR: chest X-ray; IGRA: interferon-gamma release assay; PLHIV: people living with human immunodeficiency virus; TB: tuberculosis; TBST: *Mycobacterium tuberculosis* antigen-based skin test; TPT: tuberculosis preventive treatment; TST: tuberculin skin test.

The first step of the cascade of care (first health care encounter or visit) is to identify target populations for TPT, and initiate screening for both TB disease and TB infection. This step can be implemented by health care personnel, in health facilities or at home, depending on the organization and resources of the local health system. Standard operating procedures (SOPs) and implementation protocols should be developed for systematic implementation of activities across the cascade of care. Health care personnel in all facilities must be trained to identify and screen priority populations, especially PLHIV and household contacts of TB patients. In addition to these populations, at secondary and tertiary facilities, health care personnel should also actively search for other high-priority groups – for example, transplant candidates, patients with autoimmune disease starting immunosuppressive drugs and patients with cancer starting chemotherapy – according to national policy. Mechanisms should be establishedto ensure that all those at high risk are identified; such mechanisms can include reminders in medical charts, registry books and digital tools, as long as these are regularly checked.

In regard to the first visit (i.e. the initial evaluation of individuals in target population):

- a registered nurse, a nurse aid or a community health agent should ask about TB symptoms (cough, fever, night sweats and weight loss);

- highly accurate screening tools should be used where available; they include CXR (with or without computer-aided detection), WHO-recommended rapid diagnostics or C-reactive protein for PLHIV, as specified in the WHO screening guidelines *(28)*; and
- a TB infection test should be performed – if the TB screen is positive, the person should still have TB infection testing, along with investigations for TB disease initiated on the same day, as specified in the WHO diagnostic guidelines *(31)*.

In regard to the second visit:

- it should occur 48–72 hours after the first visit, and should include reading the TB infection skin test reaction or obtaining the IGRA result; and
- it is required for all those who initially were screened positive for TB disease but in whom TB disease was excluded, and all those who initially screened negative for TB disease:
  - if the TB infection test is negative, and the person is well, they can be discharged;
  - if the person is initially symptomatic but TB disease has been satisfactorily excluded and the TB infection test is positive, then TPT can be initiated immediately (as per the algorithm in Fig. 2.1 in Chapter 2); and
  - if the person is initially screened negative for TB but the TB infection test is positive, then CXR (if not already done in the initial screening) is warranted, with medical evaluation to exclude TB disease – all these activities should be undertaken on the same day, so that TPT can be prescribed during this same visit.

Provision of integrated patient-centred care requires health services to be organized to screen and evaluate further for TB disease and initiate TB infection testing during the first visit. Also, during the second visit, services must be coordinated to deliver test results (TB infection skin test reading or IGRA results), counselling (including for parents or guardians), CXR and medical evaluation. The identification and referral of patients for TB disease screening and TB infection testing, as well as management of those with positive and negative tests, should be part of a well-established written protocol. A qualified and trained provider (usually a nurse or a nurse's aide) should administer the TB infection skin tests. A qualified phlebotomist (laboratory technician, nurse or nurse's aide) should collect the blood sample for IGRA.

The NTP or national tuberculosis reference laboratory usually determines which sites will conduct diagnostic testing, based on factors such as epidemiology, geographical considerations, testing workload, availability of qualified staff, efficiency of referral networks and patient access to services. Two tests – QFT-Plus and WANTAI TB-IGRA – can be performed in laboratories equipped with centrifuges and optic densitometers (for ELISA); however, for T-SPOT.TB, the normalization step requires that PBMCs are separated from whole blood and counted, meaning that additional equipment may be required. When considering IGRAs as the test for TB infection, trained phlebotomists are required to draw the blood sample; also required arerapid transport mechanism for samples, to ensure they reach laboratories performing IGRA tests within the timeframe specified in the package insert. There is also an option for centrifugation of blood at smaller laboratories with transport within a specified time to larger laboratory performing IGRA tests. When considering TST or TBST as the test for TB infection and to maximize accessibility, every primary health care clinic should have refrigeration for storage of TB infection skin tests, and personnel trained to administer and read TB infection skin tests.

Programmes should plan patient support and follow-up for TPT. At a minimum, this means follow-up visits during which health care providers evaluate adherence, tolerability and potential adverse events, and reinforce the importance of completing TPT. Missed visits should trigger an active follow-up for the person and treatment support. Services may also include video support or other mHealth technologies such as instant messaging, to enhance TPT completion (such support will require equipment and technical support as well as human resources for follow-up).

The fourth and last step is recording and reporting of TPT outcomes, ideally using an online information system.

4.2 Steps for programmatic implementation of testing for TB infection

The 10 main areas in implementing a new test are listed in Box 4.1. The following sections describe the steps within each of the 10 areas.

Box 4.1. Summary of key areas for implementing and scaling up tests used to detect TB infection

Area 1: Policies, budgeting and planning (Section 4.2.1)

Area 2: Regulatory approval and importation of products (Section 4.2.2)

Area 3: Equipment and materials (Section 4.2.3)

Area 4: Human resource sensitization, training and competency assessment (Section 4.2.4)

Area 5: Supply chain (Section 4.2.5)

Area 6: Procedures (Section 4.2.6)

Area 7: Digital data (Section 4.2.7)

Area 8: QA, QC and quality assessment (Section 4.2.8)

Area 9: Recording and reporting (Section 4.2.9)

Area 10: Monitoring and evaluation (Section 4.2.10)

QA: quality assurance; QC: quality control; TB: tuberculosis.

4.2.1 Area 1 – Policies, budgeting and planning

1.1 Establish a technical working group (TWG), and define roles and responsibilities

1.2 Review WHO policies and available technical and implementation guides

1.3 Define immediate and future purposes of tests to detect TB infection

1.4 Update national diagnostic algorithm and guidelines

1.5 Perform a situational analysis

1.6 Develop a costed operational plan for phased implementation or scale-up

Step 1.1 – Establish a TWG, and define roles and responsibilities

A TWG comprising representatives from all key stakeholders should be established or the function of an existing TWG should be expanded to cover tests for TB infection. The TWG is critical to guide national policy development and the implementation or scale-up of TB infection testing services of the programme. The TWG should be led by the ministry of health, with a convening role for the national programmes (e.g. TB and HIV) and the national TB reference laboratory. The TWG should be mandated to advise the ministry of health, NTP and national tuberculosis reference laboratory on national policy and development of implementation tools such as testing algorithms, SOPs and protocols, and mechanisms for clinical evaluation and linkage to appropriate care and treatment. The TWG should also develop implementation plans; monitor quality of implementation and suggest steps to address gaps in implementation across the cascade of care including implementation of new tests; and review the impact and success of the programmatic introduction of testing services. Representatives from the following key stakeholders may be invited to participate:

- Ministry of health, national programmes (e.g. NTP, HIV/AIDS programmes and noncommunicable disease programmes), national TB reference laboratory and clinical (pathology) laboratory networks;
- ministry of justice and ministry of education;
- research institutes or other organizations with experience in programme implementation research and the introduction of new diagnostic tests;
- representatives from managers of municipal or provincial health offices, clinical facilities and peripheral laboratories that will participate in the programme;
- representatives from national regulatory bodies that will approve and incorporate tests and procedures;
- representatives from data management or information technology (IT) experts and managers that enable interoperability of electronic systems; and
- other technical and implementing partners outside TB and HIV programmes (e.g. community and civil society representatives), and other UN agencies

A suitably qualified individual should lead the team; for example, an officer from the NTP. An integral component of the planning process should be defining the roles and responsibilities of members of the implementation or scaling-up team, and those of external partners and donors.

Step 1.2 – Review WHO policies and available technical and implementation guides

The TWG members should familiarize themselves with the contents of the relevant WHO policies, guidance, handbooks and reports, as well as any available implementation guides from WHO, the Global Laboratory Initiative, the Foundation for Innovative New Diagnostics (FIND) and implementing partners. Particular attention should be paid to WHO policies and recommendations on who to test, how to test (using new tests) for TB infection, the limitations of tests and the interpretation of test results.

Step 1.3 – Define immediate and future purposes of tests to detect TB infection

Programmes must clearly define the purpose, scope and intended use of the new diagnostic test for TB infection, because that has Implication for planning and resource mobilization. For example, the laboratory system or network needed to provide timely results for patient-care decisions only in people who are initiating anti-TNF treatment is quite different from that needed to conduct large-scale testing for TB infection in, for example, PLHIV, household contacts of people with bacteriologically confirmed pulmonary TB, prisoners and health care workers.

Step 1.4 – Update national diagnostic algorithm and guidelines

The TWG should undertake a review of existing national policy recommendations and diagnostic algorithms, taking into consideration the needs of patients, clinical needs, country epidemiological aspects (e.g. expected number of contacts of an index patient, expected positive results for TB infection tests), existing testing algorithms, sample referral systems where IGRAs are implemented and the feasibility of different algorithms; the TWG should then make recommendations to the ministry of health and NTP. Roles of different health care personnel should be clearly established.

The TWG should advise the ministry of health and NTP on the development of clinical guidelines. Such guidelines must provide clear guidance to clinicians, pulmonologists, infectious disease doctors, nurses and health care professionals on the algorithms, target patient populations and intended use of diagnostic tests. Also, the guidelines must give special attention to new tests; how to communicate, interpret and use test results; the options for TPT regimens; and the type of contacts that should be investigated.

The TWG should be kept functional after implementation or scale-up of the TB infection programme. New evidence is constantly emerging on tests for TB infection and risk of progression, on TPT regimens , and on monitoring the quality of testing services and linkage to care and treatment according to national guidelines.

Step 1.5 – Perform a situational analysis

Recognizing the barriers in the various steps of the cascade of care and implementing specific tailored solutions for overcoming the barriers is feasible, effective, affordable and sustainable in LMIC *(55–57)*. Digital tools have been successfully used to analyse all steps of the cascade of care and understand the main bottlenecks *(58–60)*, and a tool to evaluate the progression on the cascade of care steps is available online *(61)*. An example from Bastos et al. *(59)* is shown in Fig. 4.2. Implementation or expansion of TB infection testing using TB infection skin tests

are not expected to create major barriers *(15, 20)*. Incorporation of TBST in settings with high levels of BCG vaccination should be done with care because TBSTs are more specific than TST in such populations. In countries or settings where TST is already in place, it should be possible to implement TBST using the same systems. However, it is important to consider needs such as initial and refresher training of health care personnel in proper techniques of test administration and reading (see Areas 4 and 8), an uninterrupted supply of TB infection skin test materials and proper cold chain for transport and storage of these materials (see Area 5). Additionally, the introduction of the newer TBST may also have regulatory requirements specific to each country.

Fig. 4.2. Example of repeated cascade analyses: corrective actions taken before and after implementation, to improve retention in the TB infection cascade of care (from Brazil)
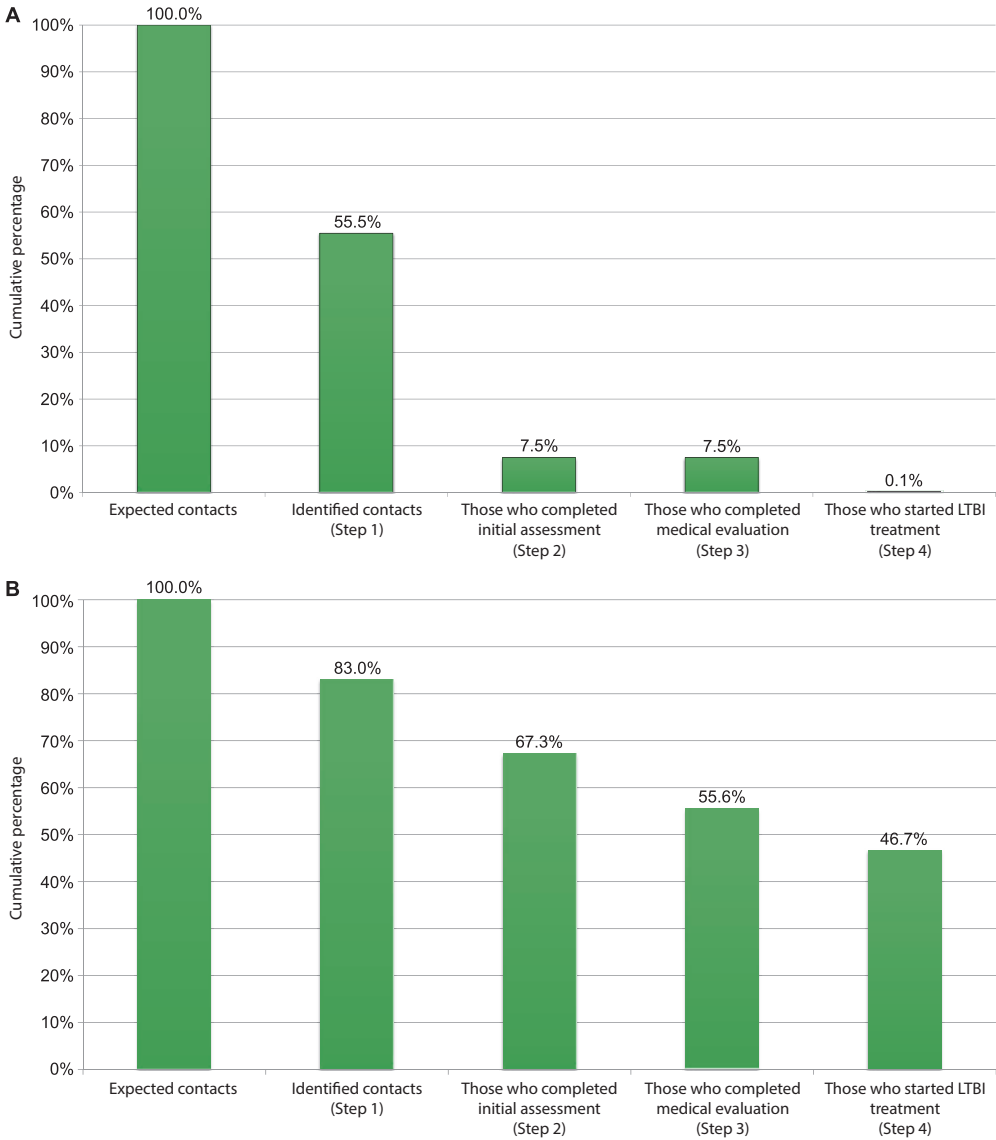
TB: tuberculosis; LTBI: latent tuberculosis infection.

In this example, solutions implemented to improve the cascade of care included sensitization and training of health care providers in primary care clinics and distribution of educational materials to health care professionals and patients.

A) Cumulative percentage of contacts of all ages retained at each step in the care cascade, streamlined evaluation phase;

B) Cumulative percentage of contacts of all ages retained at each step in the care cascade, May–October 2018.

Source: Bastos et al. (2020) *(59)*.

Implementation of IGRAs requires laboratory and network infrastructure, a functioning sample transportation system, IT capabilities, diagnostics connectivity, and availability and adequacy of SOPs. An example of a checklist for evaluating a specimen referral system is available *(62)*. Staff skills, expertise and experience should be reviewed and updated if needed (see Areas 4 and 8).

The situational assessment should also include identification of gaps in systematic recording and reporting of events across the cascade of care for TB screening and TPT. The TWG should also advise on data variables to be included in the national HMIS system, to enable quality implementation and monitoring and evaluation of services for testing for TB infection.

Step 1.6 – Develop a costed operational plan for phased implementation or scale-up

The final step in Area 1 is to develop a detailed, costed and prioritized action plan for phased programmatic implementation, with coverage targets and a timeline. Often, implementation of a new interventions must overcome potential challenges such as the cost of human resources, cost of instruments, ancillary equipment and consumables; requirements for improving or establishing the necessary laboratory and network infrastructure (e.g. a specimen transport system); the need for specialized, skilled and well-trained staff; the need for expert technical assistance; maintenance of confidentiality of patient information; and establishment of a quality assessment system (see Area 8).

Staff time: Using time and motion methodology, TST was found to be the least time-consuming step in the TB infection cascade of care. In LMIC, an average of 3.1 minutes was required for TST application, and 3.2 minutes for TST reading *(63)*. Labour time for ELISA-based IGRAs is estimated by the manufacturer to be less than 1 hour per ELISA plate; however, because of the need to incubate the test for at least 16 hours, the entire laboratory process usually takes about 24 hours *(55)*. T-SPOT.TB needs more personnel hours because this test requires PBMCs to be separated and counted initially, although results are available within 24 hours for this test as well. Given that personnel costs vary according to the setting, total costs for human resources should be estimated in each country.

Costs of tests: When estimating costs of the test, apportionment of common equipment and the laboratory area used for other tests should be estimated according to the workflow in each laboratory. This can vary widely by setting. Successful implementation of the plan will require financial and human resource commitments from the ministry of health or NTP, with possible support from implementing partners. A budget should be developed to address activities in collaboration with key partners. For skin testing, investments to maintain a cold chain for the testing material can be considered in collaboration with the national immunization programme. Additional investments to enhance capacities for a cold chain may be factored in if necessary.

4.2.2 Area 2 – Regulatory approval and importation of products

> **2.1** Complete national regulatory processes
>
> **2.2** Determine importation requirements
>
> **2.3** Conduct country validation and verification studies, as required

Step 2.1 – Complete national regulatory processes

For new technologies, the ministry of health should work closely with the relevant government authorities, manufacturers and authorized service providers to meet the requirements of the national regulatory authority. Sufficient time must be allowed for review of the application and any supplementary evidence. This is particularly applicable to the new TBST and IGRA consumables, because regulatory approval is generally provided for a specific test produced by a specific manufacturer, not for the class of the test. The regulatory pathway for approval of TBST will probably be the same as the one for TST materials. In many settings, because TB infection skin test material is injected, it is considered to be a medicine or a vaccine; therefore, the time to approval may be longer with greater requirements for data, especially for safety, and the procedures for regulatory approval need to be initiated well in advance. Regulatory approval of more than one test may improve access to tests and allow countries to choose between different tests based on test performance, as well as cost–effectiveness, feasibility and other operational aspects.

Step 2.2 – Determine importation requirements

National authorities should be consulted to determine relevant processes to be followed for importation of equipment and supplies for new tests. Countries should work closely with manufacturers and authorized service providers of equipment and consumables, to determine importation and registration requirements. Consumables for the TB infection tests (both IGRA and TBST) should be imported respecting manufacturers' recommendations for transportation and storage. As discussed previously, TB infection skin tests avoid the need for equipment, except for refrigeration (cold chain). WHO-recommended new IGRAs use the same equipment as the previously approved QFT-GIT. Countries that were already using those tests will need to plan for scale-up, but regulatory and importation processes should already be in place.

Step 2.3 – Conduct country validation and verification studies, as required

Validation studies are an essential part of the WHO review process and development of recommendations for the use of a new test. Once large-scale validation studies have been published and a test's target performance characteristics have been established, laboratories or field sites that are implementing the method do not need to repeat such large-scale studies. Small-scale verification studies to demonstrate that the laboratory can achieve the same performance characteristics obtained in the validation studies are sufficient. Countries must make their own determination on the need for verification, particularly of a new TBST.

4.2.3 Area 3 – Equipment and materials

3.1 Select, procure, install and set up equipment

3.2 Instrument verification and maintenance

3.3 Assess site readiness and ensure a safe and functional testing site

Step 3.1 – Select, procure, install and set up equipment

For TB infection skin tests, a stand-alone or medical-grade refrigerator for storing consumables (tuberculin or TB antigens), vaccines and medications must be available. Backup generators or power supply system would also be required. Tuberculin vials should be stored at 2–8 °C (35–46 °F), protected from light, in the original packaging and separated from other similar vials (to avoid confusion). Refrigerators combined with a freezer that have a single door are not suitable. Skin test consumables should not be stored in the door of the refrigerator. Temperature should be monitored using a digital thermometer with the probe inserted in a vial of biosafe glycol or a vial of glass beads. A daily log of temperature, including (where feasible) maximum and minimum temperatures, should be kept. TB infection skin test material that has been exposed to freezing temperatures (below 0 °C) or has been stored above 8 °C should be discarded *(56)*.

Other than the skin test materials, the only consumables needed are gloves, needles, syringes and cotton. Having trained staff is crucial (see Area 4).

For IGRAs, equipped laboratories are needed. Table 4.1 lists the equipment and personnel needed for tests. Tubes, needles, gloves and other material for blood sample collection are also necessary, as are pipettes and other regular laboratory supplies. For T-SPOT.TB, some special supplies are needed, including stains for PBMC counting, positive controls and cell culture media. Equipment such as centrifuges, incubators and optic densitometer also require space, which needs to be considered during planning. Instruments should be properly positioned in a clean, secure and suitable location. Most instruments will require an uninterrupted supply of power, and appropriate working and storage conditions (e.g. humidity and temperature controlled).

For QFT-Plus and WANTAI TB-IGRA, the following equipment is needed: incubator, centrifuge, plate washer, plate agitator and microplate optical density (ELISA) reader. Those are generally available in laboratories that perform other ELISA testing such as serology. The automated QFT-Plus testing instrument provides an all-in-one system from loading of the serum to obtaining the result.

For T-SPOT.TB, a biohazard level two cabinet is recommended for initial steps of handling, separating and counting PBMCs. The manufacturer also suggests use of a humidified 37 °C ±1 °C CO_2 incubator. In addition, a centrifuge and automatic microplate washer are required, and a lymphocyte counter. Some large volume laboratories have automated spot counters; otherwise, equipment for a technician to count the spots will be required. However, the T-SPOT. TB test can be re-read at any time, which can be advantageous in regions where electricity might be interrupted.

Table 4.1. Equipment and personnel needed for WHO-recommended IGRA tests *(8)*

	QFT-GIT	WANTAI TB-IGRA	QFT-Plus	T-SPOT.TB	TB infection skin tests
Equipment					
Fridge for supply storage	☑	☑	☑	☑	☑
Lymphocyte counter	✗	✗	✗	☑	✗
Incubator	☑	☑	☑	☑	✗
Centrifuge	☑	☑	☑	☑	✗
Plate washer and agitator	☑	☑	☑	☑	✗
ELISA/ELISPOT reader	☑	☑	☑	☑	✗
Personnel					
Phlebotomist	☑	☑	☑	☑	✗
Technician for incubation	☑	☑	☑	☑	✗
Technician for centrifugation or aspiration	☑	☑	☑	☑	✗
Technician for ELISA or ELISPOT	☑	☑	☑	☑	✗
Trained health professional for intradermal injection and reading	✗	✗	✗	✗	☑

ELISA: enzyme-linked immunosorbent assay; ELISPOT: enzyme-linked immunosorbent spot; IGRA: interferon-gamma release assay; QFT-GIT: QuantiFERON Gold In-Tube; QFT-Plus: QuantiFERON-TB Gold Plus; TB: tuberculosis; WHO: World Health Organization.

Step 3.2 – Instrument verification and maintenance

Before starting to use IGRA to test clinical specimens, all instruments, including centrifuges, must be documented as being "fit-for-purpose" through verification with known positive and negative materials. Instrument verification is conducted at the time of installation, after service or calibration, and after moving instruments.

To ensure quality, reproducibility and reliability, maintenance and calibration must be performed regularly. The frequency of calibration and its intervals vary for each instrument. To decide on the frequency of calibration, the recommendations of the manufacturer should be followed at a minimum. However, where the level of accuracy of the equipment is suspected to be declining, those responsible should be able to discern the problem and take the initiative to perform calibration.

Laboratory equipment calibration requires an association between measurements of a scale or accuracy made or set with the equipment to be tested and similar measurements made with the standard (i.e. equipment with known or assigned accuracy). Standards vary from country to country, depending on the type of industry. Manufacturers designate their measurement criterion and recommend the frequency and level of calibration, depending on how often the device is used and the specific application.

Instruments require regular preventive maintenance, and ad hoc servicing and maintenance. The end-user should perform regular preventive maintenance, to ensure that the instrument is performing effectively. Suppliers or authorized service providers should perform on-request maintenance, as necessary. Countries should take advantage of any available extended warranties or service contracts to ensure continued functioning of the instruments.

Instrument calibration is not necessary for performing skin tests; this gives these tests an advantage over IGRAs, although it makes careful training and supervision more important.

Step 3.3 – Assess site readiness and ensure a safe and functional testing site

Each testing site should be evaluated for readiness using a standardized checklist before testing of clinical specimens at the site begins. In addition, existing testing sites should be assessed regularly for safety and operational functionality. This includes training or assessing staff competency to apply tuberculin and read induration (see Area 4) or, in the case of IGRAs, laboratory capacity, phlebotomist availability and the ability to transport samples rapidly (i.e. on the same day) and for the laboratory to deliver results to the providers no more than 48–72 hours after blood collection for IGRA tests.

Appropriate personal protective equipment (PPE) should be used for handling blood samples, and biological waste must be disposed of safely and in accordance with regulations. Biosafety regarding occupational *M. tuberculosis* transmission is not a concern for TB infection tests. Standard precautions are necessary when handling blood or manipulating needles.

4.2.4 Area 4 – Human resource sensitization, training and competency assessment

4.1 Develop and implement a training curriculum and strategy

4.2 Sensitize health staff on the importance of a TPT programme

4.3 Assess and document the competence of staff

Step 4.1 – Develop and implement a training curriculum and strategy

A high-quality TPT programme requires skilled staff for performing the tests. Clinical staff need training in all steps of the cascade of care, including how to identify target populations eligible for TPT, provide counselling and education, perform and interpret TB infection tests, collect and transport blood samples for IGRAs, and undertake medical evaluation and CXR to determine eligibility for treatment after testing. For countries adopting IGRAs, additional training for the

laboratory tasks will be needed and similarly health care workers in the field will need to be trained in skin testing and reading of test reactions.

The TWG and national programmes need to establish clear guidelines to define the priority groups for testing (and treating), the methods and techniques for the tests, interpretation of test results and next steps after testing. Educational material developed in the local language for health care personnel and patients is useful *(59)*; examples are available online *(44, 64)*.

For TB infection skin tests, training nurses for injection and reading of induration is crucial. Simple protocols for training *(57)*, and using mobile technologies for training and QC of skin testing *(45)* may facilitate the widespread use of these tests, even in remote settings. Practical training material is available in several languages; for example, for injection *(43)* and reading of results *(65)*.

As discussed in Area 8, close QC and quality assessment of human resource competency is necessary. Digital tools (as exemplified in Area 7) may indicate which areas of training need to be strengthened.

Step 4.2 – Sensitize health staff on the importance of a TPT programme

Sensitizing health care personnel on the importance of programmatic management of TPT in reducing TB mortality and morbidity is critical to enhance acceptance and promote uptake of tests for TB infection. Both TPT and testing for TB infection should be integral parts of training on the national guidelines *(58, 59)*. In addition, engagement with professional associations for doctors and nurses, and organization of continuing medical education programmes may be required when testing intervention is introduced. Using e-learning modules may help with faster cascading of the training. Ongoing mentoring during review meetings and field visits of programme managers and field supervisors is also crucial to ensure quality of implementation of all activities across the cascade of care.

Step 4.3 – Assess and document the competence of staff

For TB infection skin tests, assessment of staff competency should be part of a cyclic process of QC and quality assessment. This can be in person or at distance (see Area 8). If the latter, training and QC can be achieved with the help of digital technologies *(45)* (see Area 8). Interventions should be undertaken when appropriate, with reassessment performed afterwards. For IGRAs, QC and quality assessment should follow standard laboratory procedures (see Area 8). However, the competence of staff to perform all steps of the cascade of care should be assessed and re-training should be offered when necessary.

4.2.5 Area 5 – Supply chain

Uninterrupted availability at all testing sites of skin test materials, or reagents and disposables for IGRAs, is essential. Careful assessment of consumption rates is necessary to avoid shortage or excessive wastage of materials according to their shelf life.

> **5.1** Review forecasting, ordering and distribution procedures
>
> **5.2** Develop procedures to monitor reagent quality and shelf life

Step 5.1 – Review forecasting, ordering and distribution procedures

The following measures will be required to ensure an uninterrupted supply of skin test materials, or IGRA reagents and disposables:

- ensuring that qualified laboratory and nursing staff have input into defining the specifications for reagents, consumables and equipment; and streamlining of importation and in-country distribution procedures to ensure sufficient shelf life of reagents and consumables, once they reach testing sites;
- careful monitoring of consumption rates, tracking of test materials with specific shelf lives and forecasting to avoid expirations or stock-outs;
- careful planning to ensure that sites have received training and that equipment has been installed ahead of the shipment of reagents;
- ongoing monitoring of all procurement and supply chain steps, to ensure that delays are minimized and that sites receive correct reagents as per the planned schedule;
- regular reassessment of purchasing and distribution strategies, to ensure that they are responsive to needs and the current situation; and
- coordination with the NTP to ensure that there is adequate drug supply to treat the cases of TB infection detected.

There has been a global shortage of tuberculin in recent years, with a direct impact on TB infection screening in countries that rely on TST *(66)*. Tuberculin is not listed among the supplies in the Global Drug Facility list; this can hamper the acquisition and distribution of pre-qualified material. This is being addressed for TBST consumables. Availability of TBST may help to overcome some of these challenges in the near future.

Step 5.2 – Develop procedures to monitor reagent quality and shelf life

The shelf life of reagents and their required storage conditions must be considered when designing a procurement and distribution system. Laboratory managers should routinely monitor reagent quality and shelf life to ensure that high-quality test results are generated. Also, the laboratory must establish SOPs for handling the reagents and chemicals used, to ensure both quality and safety.

Once the tuberculin vial has been opened, the material should be used within 30 days; thus, the date of opening should be clearly written on the label of the vial. The vial should be stored immediately after use for the day, at the prescribed temperature. Any remaining tuberculin must be discarded after 30 days. The duration varies for TBST; hence, manufacturer specifications should be followed.

For IGRAs, new-lot testing (also known as lot-to-lot verification) should be performed on new batches of reagents or test kits. Such testing usually involves testing a sample of the new materials and comparing the results to an existing lot of materials with known performance.

Where possible, new-lot testing of commercially available test kits should be performed at the central level (e.g. at the national tuberculosis reference laboratory) or regional level, to ensure that kits with test failures are not distributed. For QC, WHO recommends using positive and negative controls when testing new batches of reagents.

4.2.6 Area 6 – Procedures

> **6.1** Develop SOPs
>
> **6.2** Update clinical procedures and strengthen the clinical–laboratory interface

Step 6.1 – Develop SOPs

SOPs must be developed or adapted for TB infection skin or blood-based tests, for:

- identifying individuals for whom the test should be performed;
- collecting, processing, storing and transporting specimens to the testing laboratory;
- laboratory testing for IGRAs, and tuberculin injection and induration reading for TB infection skin tests;
- data security and confidentiality (see Area 7);
- process controls (internal QC) and external quality assessment (see Area 8);
- recording and reporting of results (see Area 9); and
- waste management.

It is essential to have a well-defined, comprehensive set of SOPs that addresses all aspects of the testing processes – from sample collection to reporting of results – as well as for skin test administration and induration size reading, because errors at any step can have a significant impact on the quality of testing. Some SOPs will rely on the manufacturer's protocols included with commercial kits whereas others will need to be developed. SOPs must be made readily available for staff and must be updated regularly, both for clinical and laboratory staff.

Step 6.2 – Update clinical procedures and strengthen the clinical–laboratory interface

A comprehensive plan to implement or scale up diagnostic testing for TB infection must address all relevant parts of the diagnostic cascade, not just what happens in the laboratory. In addition to SOPs for TB infection testing, clear clinical protocols and guidance will be needed for selecting patients to be tested, ordering tests, interpreting test results, reporting results and making patient-care decisions. Before the introduction of a new diagnostic test or any changes in an existing test, all clinical staff involved in the diagnosis and management of patients must be informed about the planned changes, and relevant training must be conducted. Information must also be shared with clinical staff at all referral sites through staff training opportunities and through use of standardized educational materials developed by the NTP.

This planning is now well accepted as part of implementation of a new laboratory test, and should be considered equally essential when implementing or scaling up TST or TBST.

4.2.7 Area 7 – Digital data

> **7.1** Develop the use of digital data and diagnostics connectivity
>
> **7.2** Develop procedures for data backup, security and confidentiality

Step 7.1 – Develop the use of digital data and diagnostics connectivity

TST and TBST results have the advantage of being provided by the clinical staff themselves. Nevertheless, the results should be adequately registered.

For IGRAs, a mechanism must be in place to deliver test results to patients and the health care provider. Results may be made available online via a secure link, and email or SMS may be used to alert the person tested or the requesting clinician that results have been issued. Digital technologies make the delivery of results quicker and at times more reliable, although print copies may also be feasible and acceptable if they are delivered in a timely fashion (i.e. within 48–72 hours of blood sample collection).

Step 7.2 – Develop procedures for data backup, security and confidentiality

In addition to the recording of the test result in the medical chart, also useful for NTP planning and organization are public health surveillance systems to record those who are eligible for the test, test results and subsequent steps. NTPs need to ensure that data are secure and backed up, especially if test results are registered in electronic systems. SOPs for regular backup and retrieval of data are required, as are policies to secure confidentiality of patient data. Antivirus, antipiracy and antihacking mechanisms should be in place, as for any other health surveillance system.

4.2.8 Area 8 – QA, QC and quality assessment

> **8.1** Implement a comprehensive QA programme
>
> **8.2** Establish and monitor QC
>
> **8.3** Develop an external quality assessment programme
>
> **8.4** Monitor and analyse quality indicators

Step 8.1 – Implement a comprehensive quality assessment programme

Most modern laboratories have a comprehensive quality management programme that includes external quality assessment and internal QC. These programmes are well developed and accepted for IGRAs, but are often lacking for TB infection skin tests. However, ensuring quality, reproducibility and reliability is as necessary for TB infection skin tests as for any other diagnostic test. Essential elements of a quality assessment system include:

- SOPs, training and documented competency assessment (Area 4) – applicable to both IGRAs and TB infection skin tests;

- instrument verification and maintenance (Area 3) – not applicable to TB infection skin tests;
- method validation or verification (Area 2) – more applicable to IGRAs;
- lot-to-lot testing (Area 5) – not applicable to TB infection skin tests;
- internal QC – applicable for both IGRAs and TB infection skin tests;
- external quality assessment – applicable for both IGRAs and TB infection skin tests; and
- quality indicator monitoring and continuous quality improvement – applicable for both IGRAs and TB infection skin tests.

Step 8.2 – Establish and monitor QC

TB infection skin tests are highly dependent on human skills for proper administration and measurement of induration. Internal QC for TB infection skin tests has been widely neglected and may be a source of error that jeopardizes care in TB infection. The necessary internal QC for skin testing can be achieved using skilled and experienced monitors, but direct monitoring and supervision will require greater investments into human resources if tests are decentralized to peripheral facilities (which is feasible and improves access for individuals). In this situation, digital tools – for example, the mTST approach developed for external quality assessment or artificial intelligence based skin test reading applications – should be used as they become available in the future (see below) (45).

IGRA tests are sensitive to both pre-analytic and analytic errors. Pre-analytic errors include inadequate blood collection, transportation and mixture in tubes; exposure of blood samples to inappropriate temperatures; and excessive waiting time before processing. These variables primarily affect ELISA-based tests that use in-tube blood stimulation, whereas the additional normalization step required for the T-SPOT.TB test can mitigate the impact of pre-analytical variables. Analytic errors include inadequate incubation (too short or too long), and errors in the detection and quantification of interferon-gamma. QC of analytic steps aims to detect errors due to test failure, environmental conditions or operator performance. QFT and other ELISA-based tests contain control tubes for negative (i.e. the nil tube) and for positive (i.e. the mitogen tube) results. Definitions of indeterminate IGRA results are detailed in Chapter 3.

For both ELISA- and ELISPOT-based tests, if excessive indeterminate, borderline or equivocal results emerge, the process should be reviewed and staff should be retrained in laboratory technical steps. Also, instruments should be checked and lot-to-lot testing of reagents is warranted in this situation. Pre-analytical steps (e.g. blood withdrawal and transportation) may need revision in case of high rates of indeterminate results or errors.

Step 8.3 – Develop an external quality assessment programme

Although not performed in laboratories, TB infection skin tests need continuous internal QC and external quality assessment. In many countries, TB infection skin test certification is required after complex training and control, but a follow-up quality assessment is not in place. mHealth technologies have been developed to allow quality assessment and QC of health care personnel performance even in remote areas (45). Instruction material is readily available.

For TST and TBST, there is a guide for reviewers performing the TB infection skin test quantitative assessment (46); there are also instructions for health care personnel on how to take mTST photos (47).

Both internal QC and external quality assessment will be particularly useful in countries where TBST will be adopted, but even for the classic tuberculin test, QC and quality assessment are needed to maximize the accuracy of TB infection testing.

IGRAs should also undergo close external quality assessment, in addition to internal QC. External quality assessment may include proficiency testing to determine the quality of the results generated at the testing site through comparison with a reference result. Re-testing of the same samples in different laboratories may also be used and is an approach that is well known by TB laboratory health care personnel. On-site supervisory visits are useful when implementing a new technology.

Step 8.4 – Monitor and analyse quality indicators

Quality improvement is a cyclic process in which data are obtained, recorded and analysed, corrective actions are undertaken and the assessment is repeated, using new data gathered after an intervention. Internal QC and external quality assessment are part of the quality improvement process and should be based on specific indicators. Quality improvement is a well-accepted part of all laboratory processes and should be an integral part of the performance of IGRAs and CXR. Ensuring that health care personnel are performing the correct techniques for administration and reading of TB infection skin tests is critical for accurate test results, although quality improvement processes are often forgotten for these tests. Quality improvement can be achieved through in-person monitoring and supervision, or virtually.

Quality improvement in TB infection care does not refer only to TB infection testing. The country should establish target indicators for all steps of the TB infection cascade of care; for example:

- how many contacts are expected per index patient (highly dependent on demographical information);
- how many of the contacts should be tested for TB infection (i.e. how much loss is acceptable);
- how many of the contacts are expected to be positive (based also on local epidemiological data); and
- how many cases are lost in further steps of the cascade?

A published report using cascade of care analyses to identify, correct and evaluate problems in Brazil provides a useful example of this health system quality improvement approach *(59)*.

4.2.9 Area 9 – Recording and reporting

> **9.1** Review and revise request for examination and reporting forms
>
> **9.2** Review and revise laboratory and clinical registers

Step 9.1 – Review and revise request for examination and reporting forms

In an ideal system, forms to request TB infection testing, record TB infection results, and request further medical evaluation and CXR are all part of an integrated electronic system – streamlining information flow and minimizing providers' time (and errors) in providing information. It

is suggested that forms should be short and simple, collecting only the most essential and actionable information.

Reporting results of TB infection testing, medical evaluation, CXR and other testing should also be streamlined, and ideally should be digitized to facilitate rapid referral and analysis. The Prevent TB mobile application and digital platform (Fig. 4.3) is expected to facilitate systematic recording and reporting as well as monitoring of data across the cascade of care for TB infection. Where paper forms are used, it is suggested that patients always be given a copy of the results.

Fig. 4.3. The Prevent TB digital platform

Source: WHO *(67)*.

IGRA results should be reported qualitatively (positive or negative) and quantitatively (TB antigen – Nil = N IU/mL) to facilitate interpretation. Importantly, the method used should be reported.

Step 9.2 – Review and revise laboratory and clinical registers

Registries in laboratories and medical records should be standardized. In countries that have adopted electronic medical records, a field for the new tests is required. WHO is currently revising its reporting framework *(68)*.

If surveillance systems incorporate new tests, then forms need to include fields for those new tests.

4.2.10 Area 10 – Monitoring and evaluation

10.1 Monitor implementation of the diagnostic test

10.2 Monitor and evaluate the impact of the diagnostic test

Step 10.1 – Monitor implementation of the diagnostic test

If a new test is implemented, the use of the test should be monitored at the implementation phase and tracked later. For TB infection skin tests – use of materials, number of tests that are not read and number of tests that show positive reactions can be monitored. For IGRAs, the number of tests performed, number of indeterminate results and number with positive results are useful to follow.

The quantity and quality of testing and reporting must be monitored. Staff at sites with an unexpectedly low or high testing or indeterminate rates, or very high frequencies of positive or negative test results, may need additional training and sensitization.

Step 10.2 – Monitor and evaluate the impact of the diagnostic test

The impact of the test on the number of patients starting TPT should be closely followed. As discussed above, the success of a programme to scale up TPT depends on the optimization of all steps of the cascade of care. Impact analyses and in-depth interviews on the perspectives of health care personnel and patients after implementation can also inform policy-makers. Digital tools (described in Area 7 and 9) may be useful to monitor patient-important outcomes (e.g. how many patients complete TPT and any long-term decrease of TB incidence) as well as gaps identified across all previous steps of the cascade of care. The consumption of TPT regimens may be a good indicator of quality testing services.

References

1. WHO consolidated guidelines on tuberculosis Module 1: Prevention – tuberculosis preventive treatment. Geneva: World Health Organization; 2020 (https://apps.who.int/iris/handle/10665/331170).

2. Houben R, Dodd PJ. The global burden of latent tuberculosis infection: a re-estimation using mathematical modeling. PLoS Med. 2016;13(10):e1002152 (https://pubmed.ncbi.nlm.nih.gov/27780211/).

3. Long R, Divangani M, Schwartzman K. Chapter 2: Transmission and pathogenesis of tuberculosis. In: Menzies D (ed), Canadian Tuberculosis Standards (8th edition). Ottawa: Canadian Thoracic Society; 2022:22–32 (https://doi.org/10.1080/24745332.2022.2035540).

4. Martinez L, Cords O, Horsburgh CR, Andrews JR. Risk of tuberculosis in children after close exposure: an individual-participant meta-analysis including 137,647 children from 46 cohort studies. Lancet. 2020;395(10228):973–84 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7289654/).

5. Gupta RK, Calderwood CJ, Yavlinsky A, Krutikov M, Quartagno M, Aichelburg MC et al. Discovery and validation of a personalized risk predictor for incident tuberculosis in low transmission settings. Nature Medicine. 2020;26(12):1941–9 (https://www.nature.com/articles/s41591-020-1076-0).

6. Campbell JR, Winters N, Menzies D. Absolute risk of tuberculosis among untreated populations with a positive tuberculin skin test or interferon-gamma release assay result: systematic review and meta-analysis. BMJ. 2020;368:m549 (https://www.ncbi.nlm.nih.gov/pubmed/32156698 https://www.bmj.com/content/bmj/368/bmj.m549.full.pdf).

7. Rapid communication: TB antigen-based skin tests for the diagnosis of TB infection (WHO-UCN-TB-2022.1). Geneva: World Health Organization; 2022 (https://apps.who.int/iris/handle/10665/352802).

8. Use of alternative interferon-gamma release assays for the diagnosis of TB infection: WHO policy statement. Geneva: World Health Organization; 2022 (https://apps.who.int/iris/handle/10665/351191).

9. Mantoux MC. La voie intradermique en tuberculinothérapie. Presse Med. 1912;20:146⬜8.

10. Stewart CJ. Skin sensitivity to human, avian and BCG PPDs after BCG vaccination. Tubercle. 1968;49(1):84–91 (http://www.ncbi.nlm.nih.gov/pubmed/5690764).

11. Menzies R, Vissandjee B. Effect of bacille Calmette-Guérin vaccination on tuberculin reactivity. Am Rev Respir Dis. 1992;145(3):621⬜5 (https://pubmed.ncbi.nlm.nih.gov/1546843/).

12. Palmer C, Edwards L. Identifying the tuberculous infected. The dual-test technique. JAMA. 1968;205(3):167–9 (http://www.ncbi.nlm.nih.gov/pubmed/5694785).

13. Anderson P, Munk M, Pollock S, Doherty T. Specific immune-based diagnosis of tuberculosis. Lancet. 2000;356(9235):1099–104 (http://www.ncbi.nlm.nih.gov/pubmed/11009160).

14. WHO consolidated guidelines on tuberculosis Module 3: Diagnosis – tests for tuberculosis infection. Geneva: World Health Organization; 2022.

15. Alsdurf H, Hill P, Matteelli A, Getahun H, Menzies D. The cascade of care in diagnosis and treatment of latent tuberculosis infection: a systematic review and meta-analysis. Lancet Infect Dis. 2016;16(11):1269 (https://pubmed.ncbi.nlm.nih.gov/27522233/).

16. Comstock G, Edwards L, Livesay V. Tuberculosis morbidity in the US Navy: its distribution and decline. Am Rev Respir Dis. 1974;110(5):572–80 (https://pubmed.ncbi.nlm.nih.gov/4429253/).

17. Horwitz O, Wilbek E, Erickson P. Epidemiological basis of tuberculosis eradication. Longitudinal studies on the risk of tuberculosis in the general population of a low-prevalence area. Bull World Health Organ. 1969;41:95–113 (https://pubmed.ncbi.nlm.nih.gov/5309087/).

18. Pai M, Kuimoto D, Jamieson F, Menzies D. Diagnosis of latent tuberculosis infection. In: Menzies D (ed), 7th ed, Canadian TB standards. Ottawa: Public Health Agency and Canadian Thoracic Society; 2013 (https://www.canada.ca/en/public-health/services/infectious-diseases/canadian-tuberculosis-standards-7th-edition.html).

19. Marais B, Gie R, Schaaf H, Hesseling A, Obihara C, Starke J et al. The natural history of childhood intra-thoracic tuberculosis: a critical review of literature from the pre-chemotherapy era. Int J Tuberc Lung Dis. 2006;8(4):392–402 (https://pubmed.ncbi.nlm.nih.gov/15141729/).

20. Bastos ML, Melnychuk L, Campbell JR, Oxlade O, Menzies D. The latent tuberculosis cascade-of-care among people living with HIV: A systematic review and meta-analysis. PLoS Med. 2021;18(9):e1003703 (https://www.ncbi.nlm.nih.gov/pubmed/34492003).

21. Comstock G. False tuberculin test results. Chest. 1975;68(3):465–9 (https://pubmed.ncbi.nlm.nih.gov/1157566/).

22. Reichman L, O'Day R. The influence of a history of a previous test on the prevalence and size of reactions to tuberculin. Am Rev Respir Dis. 1977;115:737–41 (https://pubmed.ncbi.nlm.nih.gov/404946/).

23. Edwards P. Tuberculin negative? New Eng J Med. 1972;286(7):373–4 (https://pubmed.ncbi.nlm.nih.gov/5008560/).

24. Chorba T. COVID mRNA vaccines, TST, and IGRA – follow-up: 31 August 2021 [website]. Atlanta: United States Centers for Disease Control and Prevention; 2021 (https://www.cdc.gov/tb/publications/letters/2021/covid-mrna-followUp.html).

25. WHO information note. COVID-19: considerations for tuberculosis (TB). Geneva: World Health Organization; 2021 (https://apps.who.int/iris/rest/bitstreams/1344785/retrieve).

26. Tarlo S, Day J, Mann P, Day M. Immediate hypersensitivity to tuberculin in-vivo and in-vitro studies. Chest. 1977;71(1):33–7 (https://pubmed.ncbi.nlm.nih.gov/11970/).

27. Spiteri M, Bowman A, Assefi A, Clarke S. Life threatening reaction to tuberculin testing. BMJ. 1986;293:243–4 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1341180/).

28. WHO operational handbook on tuberculosis Module 2: Screening – systematic screening for tuberculosis disease. Geneva: World Health Organization; 2021 (http://apps.who.int/iris/handle/10665/340256).

29. WHO consolidated guidelines on tuberculosis Module 3: Diagnosis – rapid diagnostics for tuberculosis detection. Geneva: World Health Organization; 2020 (https://apps.who.int/iris/handle/10665/332862).

30. WHO operational handbook on tuberculosis Module 1: Prevention – tuberculosis preventive treatment. Geneva: World Health Organization; 2020 (https://apps.who.int/iris/handle/10665/331525).

31. WHO operational handbook on tuberculosis Module 3: Diagnosis – rapid diagnostics for tuberculosis detection 2021 update. Geneva: World Health Organization; 2021 (https://apps.who.int/iris/handle/10665/342369).

32. Seibert F, Glenn J. Tuberculin purified protein derivative: preparation and analyses of a large quantity for standard. Am Rev Tuberc. 1941;44:9 (https://www.atsjournals.org/doi/abs/10.1164/art.1941.44.1.9).

33. Snider D, Farer L. Package inserts for antituberculosis drugs and tuberculins. Am Rev Respir Dis. 1985;131:809–10 (https://www.atsjournals.org/doi/abs/10.1164/arrd.1985.131.6.809).

34. Further studies of geographic variation in naturally acquired tuberculin sensitivity. Bull World Health Organ. 1955;12(1–2):63–83 (http://www.ncbi.nlm.nih.gov/pubmed/14351968).

35. Farhat M, Greenaway C, Pai M, Menzies D. False positive tuberculin skin tests – what is the absolute effect of BCG and non-tuberculous mycobacteria? Int J Tuberc Lung Dis. 2006;10(11):1–13 (https://www.ingentaconnect.com/content/iuatld/ijtld/2006/00000010/00000011/art00003).

36. Lewinsohn D, Leonard M, Lobue P. Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention clinical practice guidelines: diagnosis of tuberculosis in adults and children. Clin Infect Dis. 2017;64(2):111–5 (https://pubmed.ncbi.nlm.nih.gov/28052967/).

37. Cy-Tb package insert. Pune: Serum Institute of India; 2022.

38. Hamada Y, Kontsevaya I, Surkova E, Wang T, Liu V, Ziganshina L et al. A systematic review on the safety of novel *Mycobacterium tuberculosis* specific antigen-based skin tests for TB infection compared to tuberculin skin tests or blood-based in vitro interferon-gamma release assays. In press.

39. Comstock G, Edwards L, Philip R, Winn W. A comparison in the United States of America of two tuberculins, PPD-S and PPD-RT 23. Bull World Health Organ. 1964;31:161–70 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2555164/).

40. Diaskintest package insert. Russian Federation: Generium; 2022.

41. C-TST package insert. China: Anhui Zhifei Longcom; 2022.

42. Menzies D, Doherty T. Diagnosis of latent tuberculosis infection. In: Raviglione M (ed), Reichman and Hershfield's tuberculosis, a comprehensive international approach. New York: Informa Healthcare USA; 2006:215–63 (https://www.taylorfrancis.com/chapters/edit/10.3109/9780203908464-15/diagnosis-latent-tuberculosis-infection-dick-menzies-mark-doherty).

43. Injection – TST technique training (English) [website]. Montreal: McGill International TB Centre; 2021 (https://www.youtube.com/watch?v=tRqumpCb_Js&list=PLwoB2EX6lRZRWCw4yyYHDQAADC9QO892V&index=5).

44. Cadernos de saude publica (Reports in public health) [website]. Rio de Janeiro: Escola Nacional de Saude Publica Sergio Arouca; 2022 (http://cadernos.ensp.fiocruz.br/csp/?404).

45. Moayedi-Nia S, Barss L, Oxlade O, Valiquette C, Ly MX, Campbell JR et al. The mTST – an mHealth approach for training and quality assurance of tuberculin skin test administration and reading. PLoS One. 2019;14(4):e0215240 (https://www.ncbi.nlm.nih.gov/pubmed/30995275).

46. Guide for reviewers performing the TST quality assurance – quantitative assessment of TST injection [website]. Montreal: McGill International TB Centre; 2019 (https://www.youtube.com/watch?v=PsBTYiEAKcc&t=4s).

47. Instructions for healthcare workers: how to take mTST photos [website]. Montreal: McGill International TB Centre; 2019 (https://www.youtube.com/watch?v=7Lbt84YCsiM).

48. The WHO standard tuberculin test. Geneva: World Health Organization; 1963 (https://apps.who.int/iris/handle/10665/112241).

49. Duboczy B, Brown B. Multiple readings and determination of maximal intensity of tuberculin reaction. Am Rev Respir Dis. 1960;82:60–7 (https://pubmed.ncbi.nlm.nih.gov/13724589/).

50. Sokal J. Measurement of delayed skin-test responses. New Eng J Med. 1975;293:501–2 (https://www.nejm.org/doi/full/10.1056/NEJM197509042931013).

51. Hanson M, Comstock G. Efficacy of hydrocortisone ointment in the treatment of local reactions to tuberculin skin tests. Am Rev Respir Dis. 1968;97:472–3 (https://www.atsjournals.org/doi/abs/10.1164/arrd.1968.97.3.472).

52. QuantiFERON®-TB Gold Plus (QFT®-Plus) ELISA package insert. Germany: QIAGEN; 2019 (http://www.quantiferon.com/wp-content/uploads/2017/04/English_QFTPlus_ELISA_R04_022016.pdf).

53. WANTAI TB-IGRA package insert. Beijing: Beijing Wantai Biological Pharmacy Enterprise Co., Ltd; 2022.

54. T-SPOT.TB package insert. United Kingdom: Oxford Immunotec; 2017 (http://www.oxfordimmunotec.com/international/wp-content/uploads/sites/3/Final-File-PI-TB-US-V6.pdf).

55. Oh C, Ortiz-Brizuela E, Bastos M, Menzies D. Comparing the diagnostic performance of QuantiFERON-TB Gold Plus to other tests of latent tuberculosis infection: a systematic review and meta-analysis. Clin Infect Dis. 2020;73(5):e1116–e25 (https://pubmed.ncbi.nlm.nih.gov/33289038/).

56. Tuberculosis (TB) Prevention and Control Program. Tuberculin skin testing (TST): storage of tuberculin and need for orders to administer. Minnesota: Minnesota Department of Health; 2013 (https://www.health.state.mn.us/diseases/tb/tstfsprov.pdf).

57. Gloria L, Bastos M, Santos Junior B, Trajman A. A simple protocol for tuberculin skin test reading certification. Cad Saude Publica. 2021;37(8):e00027321 (https://www.ncbi.nlm.nih.gov/pubmed/34495087).

58. Oxlade O, Benedetti A, Adjobimey M, Alsdurf H, Anagonou S, Cook VJ et al. Effectiveness and cost-effectiveness of a health systems intervention for latent tuberculosis infection management (ACT4): a cluster-randomised trial. Lancet Public Health. 2021;6(5):e272–e82 (https://www.ncbi.nlm.nih.gov/pubmed/33765453).

59. Bastos ML, Oxlade O, Benedetti A, Fregonese F, Valiquette C, Lira SCC et al. A public health approach to increase treatment of latent TB among household contacts in Brazil. Int J Tuberc Lung Dis. 2020;24(10):1000–8 (https://www.ncbi.nlm.nih.gov/pubmed/33126931).

60. Barss L, Moayedi-Nia S, Campbell JR, Oxlade O, Menzies D. Interventions to reduce losses in the cascade of care for latent tuberculosis: a systematic review and meta-analysis. Int J Tuberc Lung Dis. 2020;24(1):100–9 (https://pubmed.ncbi.nlm.nih.gov/32005312/).

61. ACT4 registry analysis tools [website]. Quebec: McGill University; 2022 (https://www.mcgill.ca/tb/act4-registry-analysis-tools).

62. GLI specimen referral toolkit [website]. Geneva: Stop TB Partnership; 2019 (https://www.stoptb.org/gli-guidance-and-tools/gli-specimen-referral-toolkit).

63. Alsdurf H, Oxlade O, Adjobimey M, Ahmad Khan F, Bastos M, Bedingfield N et al. Resource implications of the latent tuberculosis cascade of care: a time and motion study in five countries. BMC Health Serv Res. 2020;20(1):341 (https://www.ncbi.nlm.nih.gov/pubmed/32316963).

64. Training slides for tuberculin skin testing (TST) [website]. Montreal: McGill International TB Centre; 2022 (https://www.mcgill.ca/tb/files/tb/tuberculin_skin_testing_tst_technique_training_feb202018_english.pdf).

65. Reading – TST technique training (English) [website]. Montreal: McGill International TB Centre; 2021 (https://www.youtube.com/watch?v=xsV7oHBdMEs&list=PLwoB2EX6lRZRWCw4yyYHDQA ADC9QO892V&index=4).

66. Tebruegge M, Buonsenso D, Brinkmann F, Noguera-Julian A, Pavic I, Arbore AS et al. European shortage of purified protein derivative and its impact on tuberculosis screening practices. Int J Tuberc Lung Dis. 2016;20(10):1293–9 (https://www.ncbi.nlm.nih.gov/pubmed/27725037).

67. Preventing TB [website]. Geneva: World Health Organization; 2022 (https://www.who.int/activities/preventing-tb).

68. Definitions and reporting framework for tuberculosis – 2013 revision. Geneva: World Health Organization; 2013 (https://apps.who.int/iris/handle/10665/79199).

Annex 1. Skin tests for tuberculosis infection – detailed description

This annex provides step-by-step procedures for administering and reading two types of skin test for tuberculosis (TB) infection: the tuberculin skin test (TST) and *Mycobacterium tuberculosis* antigen-based skin tests (TBSTs). The photographs used in this annex were kindly provided by Dr Richard Menzies and colleagues.

A1.1 – Test administration

Step 1. TB Screening

In an integrated and person-centred TB infection cascade of care, the first encounter with a provider will include initiation of both screening for TB disease and testing for TB infection. Just before administration of the TST or TBST, TB disease screening should be done; it should include a TB symptom check and preferably where available use more sensitive tools recommended by WHO such as CXR (with or without computer-aided detection), WHO-recommended rapid diagnostics or C-reactive protein for PLHIV,. If a contact has clinical features that suggest TB disease, then that person should be referred immediately for further diagnostic evaluation, which should be done on the same day.

Where a contact has no symptoms or has only non-TB-related respiratory symptoms (e.g. a sore throat or rhinorrhoea, with a duration of only a few days), then TB infection testing can be initiated, and these symptoms can be re-evaluated 48–72 hours later. It is important to aim to minimize loss along the TB infection care cascade. The decision to immediately evaluate an individual with symptoms, or to perform TB infection testing and re-evaluate symptoms at the time of reading, is a matter of clinical judgement.

Step 2. Explain the administration of TST or TBST

Signed informed consent is not considered necessary for TB infection testing because this is part of routine care; however, the person being tested should understand the procedures and agree to them. In cases where a child is administered the test, consent by a parent or guardian is required and the presence of the parent or guardian may be necessary. The provider should take time to explain the test procedures and emphasize the need to return for test reading within 72 hours. The provider should verify that the person can return within this time frame for the test to be read; the provider should also respect the individual's confidentiality and privacy.

If an mHealth approach is being used to measure the size of swelling immediately after intradermal injection as a quality control (QC) procedure (mTST) *(1)* or similar) the provider should explain to the patient that photos will be taken of the injection site immediately after the injection.

Step 3. Prepare for the administration of TST or TBST

It is important that the person undergoing the TB infection test is comfortable while the test is being administered. The person should be seated with their arm supported on a flat surface, such as the corner of a table, facing the provider who will administer the TB infection test. Where possible, the TB infection test should be administered in a private room, with only the person being tested in attendance (family members are appropriate).

Step 4. Select site for injection

TST and TBST tests are generally administered on the inner aspect of the forearm, about 10 cm below the elbow (middle to upper third of the forearm). The area to be injected should be free of recent cuts, burns or other injuries, and not affected by a rash or eczema. The area should also be free of scarring, particularly keloids. Skin disease or scars may interfere with the injection and proper reading of the result; also, they may cause greater discomfort from the test. The presence of tattoos is not a problem unless erythema also needs to be measured (for C-TST only). If there are such skin problems, then another site should be selected for injection.

Step 5. Prepare the syringe

Draw up the test dose in a 1 mL syringe that has markings to indicate each 0.1 mL (or smaller units). It is important to eliminate air from the syringe, to prevent accidentally injecting air into the patient and to ensure that the full test dose is administered. The syringe should be prepared just before the injection; older studies with standard purified protein derivative (PPDS) demonstrated reduced sensitivity if syringes were prepared more than 20 minutes before injection *(2)*. If the mTST QC programme or similar is in place, the smartphone or digital camera should be prepared, so that the provider is ready to take photos immediately after the injection.

Step 6. Clean the injection site

Opinion varies on how best to clean the injection site. Alcohol swabs may be used, but this may cause greater pain unless the alcohol is allowed time to dry completely (it is important not to blow on the site, because this will reinfect the area). Allowing the alcohol to dry completely is especially important when administering the test to a child. Cleaning the area with sterile saline or water is adequate and will cause less stinging if the liquid has not fully dried at the time of injection.

A topical anaesthetic should not be applied, because this can sensitize the skin. There have been case reports of induration resulting from topical anesthetics *(2)*, and that induration will be mistaken for a positive test at the time of reading.

Step 7. Administer the injection

All TB infection skin tests (TST or TBST) are administered using the Mantoux method of intradermal injection. This method of administration was first described for TST many decades ago *(3)*, and the manufacturers of the three TBSTs have all described this same method in their product monographs *(4–6)*. The material must not be injected subcutaneously because this makes it more difficult to measure the result and can even lead to false negative results.

A needle (25 gauge to 27 gauge) is placed on the syringe, which is then laid flat on the skin with the bevelled side upward. The provider then slides the needle under the skin (the tip of the needle is often visible even when it is intradermally). The material is then slowly injected (over 2–3 seconds). In cases where the test material runs out initially, the needle should be pushed in a little deeper. A small induration or papule or bleb ("weal") should form. This bleb should be at least 7 mm in diameter *(1)*. When the injection is finished, the needle should be withdrawn.

Step 8. After the injection

After the injection, there should be a small induration or bleb on the skin. If there is a little bleeding, this can be wiped away. There is no need to cover the injection site with a dressing or bandage, and no need to mark the spot (having a large circle or an X on the forearm may be stigmatizing, particularly for children).

Step 9. Take photographs (for mHealth – if applicable)

If an mHealth QC programme is in place, then the provider should take several photographs of the injection site. The syringe should be placed with gradation markings visible, 2 cm away from the injection site (towards the elbow). Once the patient has left, the best three photographs should be sent to the supervisor.

Step 10. Provide post-TST care

The patient should remain seated in the clinic area for 10–15 minutes. This is for surveillance in case of allergy or anaphylaxis, which is rare (1 per million) (25), or vasovagal fainting, which is much more common than anaphylaxis and can result in injury from an unprotected fall. If an individual feels faint during or after injection, then ensure that the person is protected from falling and immediately record vital signs, particularly blood pressure and heart rate. If the

heart rate is rapid and the blood pressure low, and particularly if there are any other signs of anaphylaxis, provide care for a potential anaphylactic reaction. If the heart rate is low (<60 beats per minute), then this is much more likely to be a simple vasovagal faint. Position the patient with their head down and use other manoeuvres effective for vasovagal reaction.

At the time of discharge, educate the patient about care of the injection site; in particular, not to scrub vigorously when washing or to scratch if there is significant itching. If there is any blistering or pain, advise the patient that they can use cold compresses and take nonsteroidal anti-inflammatories for relief.

Step 11. Arrange for the result to be read

Remind the patient of the need to return 48–72 hours later for the result to be read and provide them with an appointment (with date and time) to come for the reading. Flexibility is important to accommodate patient schedules. Ideally, the reading should be scheduled after 48 hours, so that if the patient is unable to come, the reading can be rescheduled for the next day and still fall within the 72-hour maximum window for reading. The person tested should be aware that the reading is not valid if more than 72 hours have elapsed since administration.

Step 12. Record the result and document details

In the patient's medical record or on specific forms or registries, as appropriate, it is important to record the following details regarding the test administration:

- name of person administering the TST or TBST;
- date and time of administration;
- product used and lot number;
- date on which the product vial was opened (if vials are used over multiple days, based on manufacturer instructions);
- site of injection;
- whether a bleb was seen, and any bleeding or leakage of test fluid;
- any adverse event – if the patient had hypotension or loss of consciousness, it is crucial to document whether this was due to vasovagal reaction or anaphylaxis; and
- date and time of appointment for reading.

A1.2 – Reading of TST or TBST result

Step 1. Seeing the patient

Patients should be seen as quickly as possible after they arrive for the reading of their TST or TBST. Reading of the test takes less than 3 minutes on average *(7)*. If a person must wait a long time simply for the result to be read, this may discourage other contacts in the same household from coming forward for testing or reading, and may discourage the same person from having a re-test, if needed.

Step 2. Re-check symptoms

If the person had non-TB respiratory symptoms at the time the test was administered, then these symptoms should be re-checked at the time of reading. If the symptoms are less severe or have resolved, then this individual can be considered to have "no symptoms" in the algorithm. However, if symptoms have not improved – and particularly if the symptoms have worsened or are suggestive of TB (e.g. fever or night sweats) – the individual should be considered to have symptoms for investigation. In such cases, the result of the TST or TBST should be read and recorded, but the person should be referred promptly for medical evaluation, including chest X-ray (CXR) – if not already done – and other testing as appropriate, regardless of the result of the test for TB infection.

Step 3. Place and position of patient for reading

Reading should be done in a private room (wherever possible), out of view of all other individuals (although family members can attend, as appropriate). For optimal measurement, the patient should be seated with the arm supported.

Step 4. Inspection of injection site

The site of the injection for the TST or TBST should be carefully inspected. If there is blistering, skin breakdown or lymphadenitis these should be recorded because they are considered to be strong positive reactions for all TB infection skin tests.

Erythema or redness is an indicator of potential induration (if erythema is present, then induration may be present). However, erythema does not need to be measured for TST (3), or for Cy-Tb (6), and only needs to be measured for Diaskintest if there is no induration (5). For C-TST, erythema should be demarcated and measured (4).

Induration or firm swelling can be detected visually or by palpation.

If there is no redness and no visible or palpable induration, then the test is negative and the result is marked as "0 mm". Likewise, if there is erythema but no obvious swelling, and on light palpation there is no evidence of induration, then the induration can be considered negative and "0 mm" can be recorded. If any swelling or induration can be palpated, then this should be demarcated (see Step 5) and measured.

If an mHealth QC programme is in place, then photographs of the injection site should be taken.

Step 5. Demarcation of induration

For TST, Cy-Tb and Diaskintest, the induration is measured (3, 5, 6). For C-TST, both induration and erythema are measured, and the largest of the two is used for clinical management (4).

To demarcate the edges of the induration, the ballpoint pen method can be used (8). With this method, a ballpoint pen tip is pushed gently against the skin at a 45-degree angle towards the site of the injection. If there is a firm and distinct induration, the ballpoint pen tip will consistently stop at the margins. This procedure is repeated several times from different directions around the injection site. If there is no visible or palpable induration, it is not necessary to use a ballpoint pen; the result can be marked as "0 mm".

Large reactions can be painful, and it is not necessary to insist on demarcating and measuring large painful indurations. These can be simply marked as "greater than 15 mm" or "greater than 20 mm" and any blistering or skin necrosis noted.

Step 6. Measurement

Once the edges of the induration (or, in the case of C-TST, the erythema) have been demarcated, then the diameter of the induration should be measured. For Cy-Tb and TST, the transverse diameter is measured and recorded in millimetres *(3, 6)*. For the C-TST, the transverse and longitudinal diameters of erythema and induration are measured, and the average of each is recorded *(4)*. The largest of these two will be taken as the clinically relevant information. For the Diaskintest, the largest diameter of the induration in any dimension is recorded *(5)*.

Measurement of the size of reaction is often subject to rounding error, because readers tend to group readings at 5, 10 and 15 mm. To avoid this error, it is a good practice to use machinist calipers or tailor calipers.

Step 7. Post-TST care

If the patient has blistering or skin breakdown, then it is important to prevent secondary infection. The area should be carefully cleaned and covered with a dry dressing. Patients should be instructed not to scratch the area.

Topical steroids should not be used, because these were shown to be ineffective in placebo-controlled trials *(9)*. Subcutaneous injection of steroids under the induration may be more effective, but conservative management with cold compresses and dry dressings to cover the site is usually sufficient and prescription of oral analgesics (aspirin or acetaminophen/paracetamol).

Step 8. Management of results

If the test is negative (as per Chapter 3 of the main text) and the patient is asymptomatic (or if symptoms are resolving), then the patient can be discharged. In some settings, close contacts will have a second TB infection test 8 weeks after the end of exposure if an initial test is negative – particularly in those with very recent exposure or concomitant viral infection. In this case, an appointment should be made for administration of the second test.

If the test is positive the person should be referred immediately for medical evaluation and CXR. In a well-organized person-centred care cascade, medical evaluation (including CXR) is available at the same site and on the same day. This minimizes delays between the first identification of a contact and the initiation of appropriate therapy for TB disease or infection.

Step 9. Recording and registration

The TST or TBST reading should be recorded in the patient's medical record or registries (or both); additional information may be recorded, depending on the setting and programme organization.

The following should be recorded:

- date and time of reading;
- product used and lot number;
- size of the induration in millimetres (transverse, maximal or average diameter, depending on the TST or TBST);
- for C-TST, the average diameter of erythema should also be recorded, and for Diaskintest, erythema (but only if there is no induration); for the TST and Cy-Tb, erythema does not need to be recorded;
- presence of blistering, skin necrosis, lymphangitis or lymphadenopathy; and
- for those with positive tests – disposition: date and time of follow-up for medical evaluation and CXR; for those with negative tests, date and time of follow-up appointment, if appropriate.

Certain articles provide useful general information on skin tests for TB infection *(10, 11)*.

References for Annex 1

1. Moayedi-Nia S, Barss L, Oxlade O, Valiquette C, Ly MX, Campbell JR et al. The mTST – an mHealth approach for training and quality assurance of tuberculin skin test administration and reading. PLoS One. 2019;14(4):e0215240 (https://www.ncbi.nlm.nih.gov/pubmed/30995275).

2. Menzies D, Doherty T. Diagnosis of latent tuberculosis infection. In: Raviglione M (ed), Reichman and Hershfield's tuberculosis, a comprehensive international approach. New York: Informa Healthcare USA; 2006:215–63 (https://www.taylorfrancis.com/chapters/edit/10.3109/9780203908464-15/diagnosis-latent-tuberculosis-infection-dick-menzies-mark-doherty).

3. The WHO standard tuberculin test. Geneva: World Health Organization; 1963 (https://apps.who.int/iris/handle/10665/112241).

4. C-TST package insert. China: Anhui Zhifei Longcom; 2022.

5. Diaskintest package insert. Russian Federation: Generium; 2022.

6. Cy-Tb package insert. India: Serum Institute; 2022.

7. Alsdurf H, Hill P, Matteelli A, Getahun H, Menzies D. The cascade of care in diagnosis and treatment of latent tuberculosis infection: a systematic review and meta-analysis. Lancet Infect Dis. 2016;16(11):1269 (https://pubmed.ncbi.nlm.nih.gov/27522233/).

8. Sokal J. Measurement of delayed skin-test responses. New Eng J Med. 1975;293:501–2 (https://www.nejm.org/doi/full/10.1056/NEJM197509042931013).

9. Hanson M, Comstock G. Efficacy of hydrocortisone ointment in the treatment of local reactions to tuberculin skin tests. Am Rev Respir Dis. 1968;97:472–3 (https://www.atsjournals.org/doi/abs/10.1164/arrd.1968.97.3.472).

10. Lewinsohn D, Leonard M, Lobue P. Official American Thoracic Society/Infectious Diseases Society of America/Centers for Disease Control and Prevention clinical practice guidelines: diagnosis of tuberculosis in adults and children. Clin Infect Dis. 2017;64(2):111–5 (https://pubmed.ncbi.nlm.nih.gov/28052967/).

11. Campbell J, Pease C, Daley P, Pai M, Menzies D. Diagnosis of tuberculosis infection. In: Menzies D (ed), Canadian tuberculosis standards – 8th edition. Ottawa: Canadian Thoracic Society; 2022:49–65 (https://www.tandfonline.com/doi/full/10.1080/24745332.2022.2036503).

Annex 2. Interferon-gamma release assays – detailed description

This annex provides step-by-step procedures for administering and reading three interferon-gamma release assays (IGRAs) for tuberculosis (TB): QFT-Plus, WANTAI TB-IGRA and T-SPOT.TB.

A2.1 QFT-Plus – step by step

Step 1. TB screening

The first step is to screen for TB, as outlined in Step 1 for tuberculin skin test (TST) or *Mycobacterium tuberculosis* antigen-based skin test (TBST) in Annex 1.

Step 2. Explain the administration of QFT-Plus

It is important to ensure that the patient understands the procedures and agrees to them. The patient should be informed of the consequence of being tested, and should understand the result and what they will need to do after receiving the results.

Step 3. Collect blood and handle appropriately (up to incubation)

There are various options for blood collection for the QFT-Plus *(1)*.

Option A: direct draw into QFT-Plus blood collection tubes

One option is to draw the blood directly into QFT-Plus blood collection tubes:

- Tubes should be labelled appropriately, with each tube identifiable by its label once the cap is removed, and the time and date of blood collection included on the label. It is also important to make sure that the tubes are kept at room temperature (17–25 °C) at the time of blood collection.
- A trained phlebotomist should collect 1 mL of blood by venepuncture directly into each of the blood collection tubes for each patient, ensuring that the correct volume is drawn – the validated range is 0.8–1.2 mL (Fig. A2.1.1). Underfilling or overfilling the tube with an amount outside the validated range may lead to erroneous results.

**Fig. A2.1.1. Direct draw into QFT-Plus blood collection tubes**

See video at:

https://www.quantiferon.com/us/products/quantiferon-tb-gold-plus-us/laboratory-resources/training-videos-labs/

- Once the tubes have been filled, they should immediately be shaken 10 times, just firmly enough to ensure that the entire inner surface of the tube is coated with blood, to dissolve antigens on the tube walls. Shaking too vigorously should be avoided as it may disrupt the gel and lead to aberrant results.
- Following shaking, the tubes must be transferred to an incubator set at 37 °C (±1 °C) as soon as possible (and within 16 hours of collection). If tubes are not incubated directly after blood collection and shaking, they should be inverted 10 times, to mix them, before being incubated at 37 °C.
- The QFT-Plus blood collection tubes should be incubated upright at 37 °C (±1 °C) for 16–24 hours. The incubator does not need CO_2 or humidification. This process is shown in Fig. A2.1.2.

Fig. A2.1.2. Process for direct draw into QFT-Plus blood collection tubes
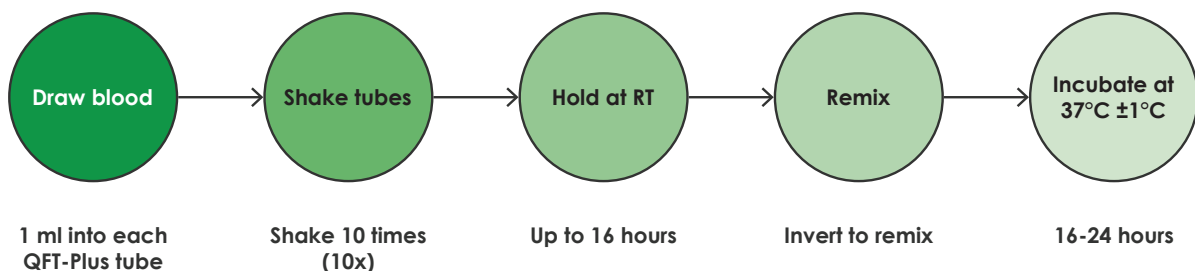
RT: room temperature.
Source: product insert (1).

Option B: blood collection into a single lithium heparin tube followed by transfer to QFT-Plus blood collection tubes

Another option is for blood to be collected in a single blood collection tube containing lithium heparin as the anticoagulant and then transferred to a QFT-Plus blood collection tube:

- Only lithium heparin should be used as a blood anticoagulant, because other anticoagulants may interfere with the assay.

- Tubes should be labelled appropriately, as for the direct draw option above.
- A trained phlebotomist should fill a lithium heparin blood collection tube (minimum volume 5 mL) (Fig. A2.1.3), then invert the tube several times to dissolve the heparin.

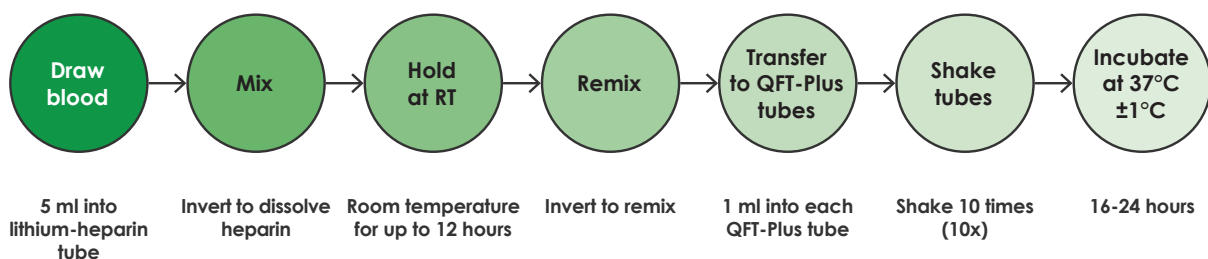## Fig. A2.1.3. Blood collection into a single lithium heparin tube

See video at:

https://www.quantiferon.com/us/products/quantiferon-tb-gold-plus-us/laboratory-resources/training-videos-labs/

There are different options for hold time and temperature for lithium heparin tubes before transfer to QFT-Plus blood collection tubes for incubation.

Option A: room temperature storage and handling of lithium heparin tube

- In this option, the blood collected in the lithium heparin tube is maintained at room temperature (17–25 °C) for no more than 12 hours from the time of collection, before being transferred to a QFT-Plus blood collection tube and then incubated (Fig. A2.1.4).

Fig. A2.1.4. Draw into lithium heparin tube and hold at room temperature

| Draw blood	Mix	Hold at RT	Remix	Transfer to QFT-Plus tubes	Shake tubes	Incubate at 37°C ±1°C
5 ml into lithium-heparin tube	Invert to dissolve heparin	Room temperature for up to 12 hours	Invert to remix	1 ml into each QFT-Plus tube	Shake 10 times (10x)	16-24 hours

RT: room temperature.

Source: product insert *(1)*.

Option B: refrigerated storage and handling of lithium heparin tube

- In this option, blood drawn into a lithium heparin tube may be held at room temperature (17–25 °C) for up to 3 hours after blood collection, then refrigerated (2–8 °C) for up to 48 hours.
- After refrigeration, the lithium heparin tube must equilibrate to room temperature (17–25 °C) before the sample is transferred to QFT-Plus blood collection tubes.
- Following transfer of the sample, the QFT-Plus blood collection tubes should be placed in the 37 °C incubator within 2 hours.

If there is a delay between transfer to QFT-Plus blood collection tubes and incubation at 37 °C, the tubes should be mixed 10 times (by inversion) before being incubated at 37 °C. Total time from blood draw to incubation in QFT-Plus blood collection tubes should not exceed 53 hours (Fig. A2.1.5).

Fig. A2.1.5. Draw into lithium heparin tube and hold at 2–8 °C
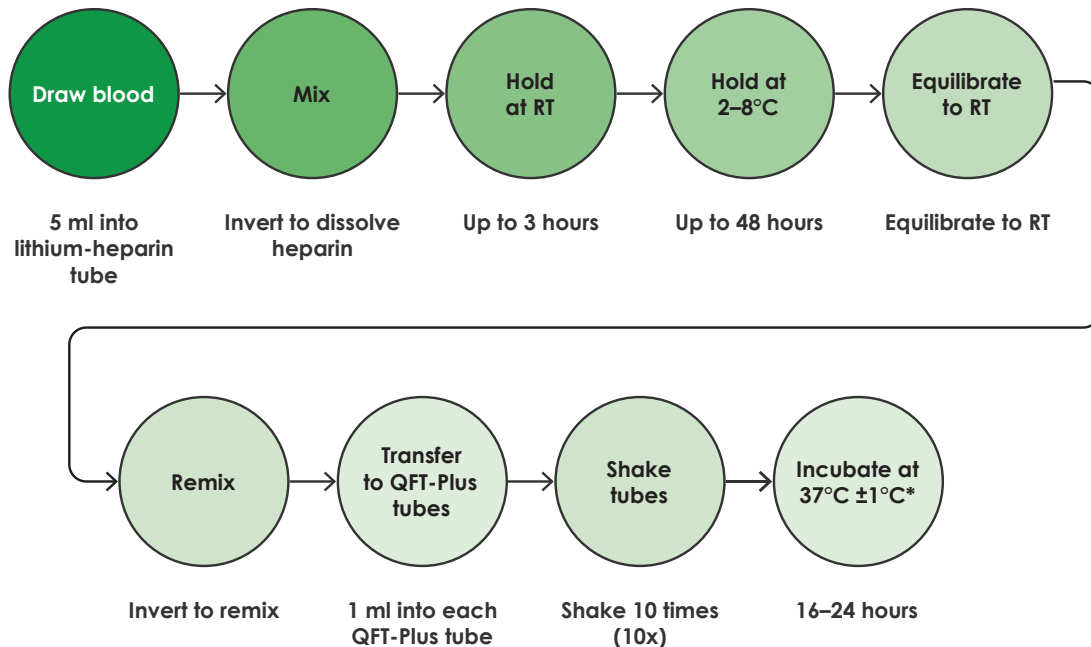
RT: room temperature.
Source: product insert *(1)*.

- Transfer of blood specimen from a lithium heparin tube to QFT-Plus blood collection tubes:
  - Each QFT-Plus blood collection tube should be labelled appropriately, ensuring that each of the four tubes (nil, mitogen, TB1 and TB2) is identifiable by its label or other means once the cap is removed.
  - The sample must be evenly mixed by gentle inversion before it is dispensed into QFT-Plus blood collection tubes. Also, the dispensing should be performed aseptically, ensuring appropriate safety procedures when removing the caps from the four QFT-Plus blood collection tubes and adding 1 mL of blood to each tube.
  - The tube caps should be replaced securely and the contents mixed as described below.
- Mixing of samples:
  - Immediately after the QFT-Plus blood collection tubes have been filled, the tubes should be shaken 10 times, just firmly enough to make sure that the entire inner surface of the tube is coated with blood, to dissolve antigens on tube walls.
  - Overly vigorous shaking should be avoided because it may disrupt the gel and lead to aberrant results.
- After mixing, the tubes must be transferred to a 37 °C ±1 °C incubator within 2 hours. If there is a delay between transfer to QFT-Plus blood collection tubes and incubation at 37 °C, the tubes should be mixed 10 times (by inversion) before being incubated at 37 °C.
- The QFT-Plus blood collection tubes should be incubated upright at 37 °C ±1 °C for 16–24 hours. The incubator does not require CO_2 or humidification.

Step 4. Centrifugation

After incubation, tubes are centrifuged at 2000–3000 relative centrifugal force (RCF). The supernatant is then extracted (the manufacturer notes that this step can be done without centrifugation, but care must be taken not to aspirate any red blood cells).

Step 5. Enzyme-linked immunosorbent assay

The enzyme-linked immunosorbent assay (ELISA) involves several steps of mixing reagents with the supernatant. This mix is then incubated for 2 hours, then the plate is washed, more reagents are added and there is another 30 minutes of incubation. Finally, the concentration of interferon-gamma is estimated from the optical density, measured using an optical density plate reader.

Materials and supplies provided by manufacturer

The product insert *(1)* explains that two components are provided by the manufacturer: the blood collection tubes (nil, mitogen, TB1 and TB2), and the ELISA kit reagents. QFT-Plus uses two types of collection tubes:

- QFT-Plus blood collection tubes, for use between sea level and 810 m: nil tubes (grey cap with white ring), mitogen tubes (purple cap with white ring), TB1 tubes (green cap with white ring) and TB2 tubes (yellow cap with white ring); and
- high altitude (HA) QFT-Plus blood collection tubes, for use between 1020 m and 1875 m: HA nil tubes (grey cap with yellow ring), HA mitogen tubes (purple cap with yellow ring), HA TB1 tubes (green cap with yellow ring) and HA TB2 tubes (yellow cap with yellow ring).

The QFT-Plus blood collection tubes should be stored at 4–25 °C. The ELISA kit reagents should be refrigerated but not frozen, and enzyme substrate solution should always be protected from direct sunlight. Other materials required but not provided are listed below. All instruments used in the procedure must be calibrated according to the manufacturer's recommendations.

Equipment needed in the laboratory

The following equipment is required for the QFT-Plus:

- 37 °C ±1 °C incubator (with or without CO_2);
- centrifuge capable of centrifuging the blood tubes at least to 3000 RCF (*g*);
- microplate shaker capable of speeds of 500–1000 rpm;
- microplate washer – for safety in handling plasma samples, an automated washer is recommended;
- microplate reader fitted with 450 nm filter and 620–650 nm reference filter;
- variable speed vortex;
- timer;
- graduated cylinder (1 L or 2 L); and
- reagent reservoirs.

Supplies needed in the laboratory

The following supplies are required for the QFT-Plus:

– calibrated variable-volume pipettes for delivery of 10 μL to 1000 μL, with disposable tips;
– calibrated multichannel pipette capable of delivering 50 μL to 100 μL, with disposable tips;
– deionized or distilled water, 2 L; and
– optional: 1 mL microtubes with caps in 96-well format racks or uncoated microplates with plastic seals for plasma storage (22 patient samples per rack or plate).

Personnel needed

For blood draws, a phlebotomist is required initially; this can be a nurse, laboratory technician, physician or other allied health professional.

If the blood is drawn directly into the QFT-Plus tube, initial handling is minimal because the tubes are placed directly in the incubator.

After incubation, personnel involved in performing QFT-Plus should be qualified laboratory technicians who are trained in ELISA techniques. Substantial staff time is required for the centrifugation, pipetting and mixing of reagents and samples; second incubation; further handling, washing and mixing; third incubation; and, finally, the actual ELISA measuring (which also involves setting up a standard curve).

Training, monitoring and quality control

Staff require initial training and ongoing supervision, monitoring and evaluation for quality control (see Chapter 4). The accuracy of test results depends on the creation of an accurate ELISA standards curve; therefore, the results from the standards must be examined before test sample results can be interpreted.

Precautions

Laboratory technicians should always wear personal protective equipment (PPE). QFT-Plus is for in vitro diagnostic use only. Failure to adhere to the manufacturer's instructions may lead to an incorrect interpretation of the results.

Interpretation of QFT-Plus

The manufacturer recommends that QFT-Plus results are interpreted based on the relative concentrations of interferon-gamma in the positive and negative controls and the TB antigen tubes *(1)*. The concentrations are calculated based on the optic density measured at the same time from wells containing known concentrations of interferon-gamma from dilutions of a standard solution. Although results are quantitative, the manufacturer recommends that qualitative results are reported to the provider (i.e. positive, negative or indeterminate) and that approval is sought from regulatory agencies for qualitative reporting (Table A2.1.1 and Fig. A2.1.6).

Table A2.1.1. Interpretation of QFT-Plus test results

Nil (IU/mL)	TB1 minus nil (IU/mL)	TB2 minus nil (IU/mL)	Mitogen minus nil (IU/mL)[a]	QFT-Plus result	Report or interpretation
≤8.0	≥0.35 and ≥25% of nil	Any value	Any value	Positive[b]	*M. tuberculosis* infection likely
	Any value	≥0.35 and ≥25% of nil			
	<0.35 or ≥0.35 and <25% of nil	<0.35 or ≥0.35 and <25% of nil	≥0.50	Negative	*M. tuberculosis* infection NOT likely
	<0.35 or ≥0.35 and <25% of nil	<0.35 or ≥0.35 and <25% of nil	<0.50	Indeterminate[c]	Likelihood of *M. tuberculosis* infection cannot be determined
>8.0[d]	Any value				

ELISA: enzyme-linked immunosorbent assay; IU: international units; *M. tuberculosis*: *Mycobacterium tuberculosis*; TB: tuberculosis.

[a] Responses to the mitogen positive control (and occasionally to the TB antigen) can be outside the range of the microplate reader; this does not affect the test results. Values >10 IU/mL are reported by the QFT-Plus software as >10 IU/mL.

[b] Where *M. tuberculosis* infection is not suspected, initially positive results can be confirmed by re-testing the original plasma samples in duplicate in the QFT-Plus ELISA. If repeat testing of one or both replicates is positive, the test result is considered positive.

[c] Refer to "Troubleshooting guide" in the package insert for possible causes.

[d] In clinical studies, less than 0.25% of subjects had interferon-gamma levels >8.0 IU/mL for the nil value.

Source: product insert *(1)*.

Fig. A2.1.6. Interpretation of QFT-Plus
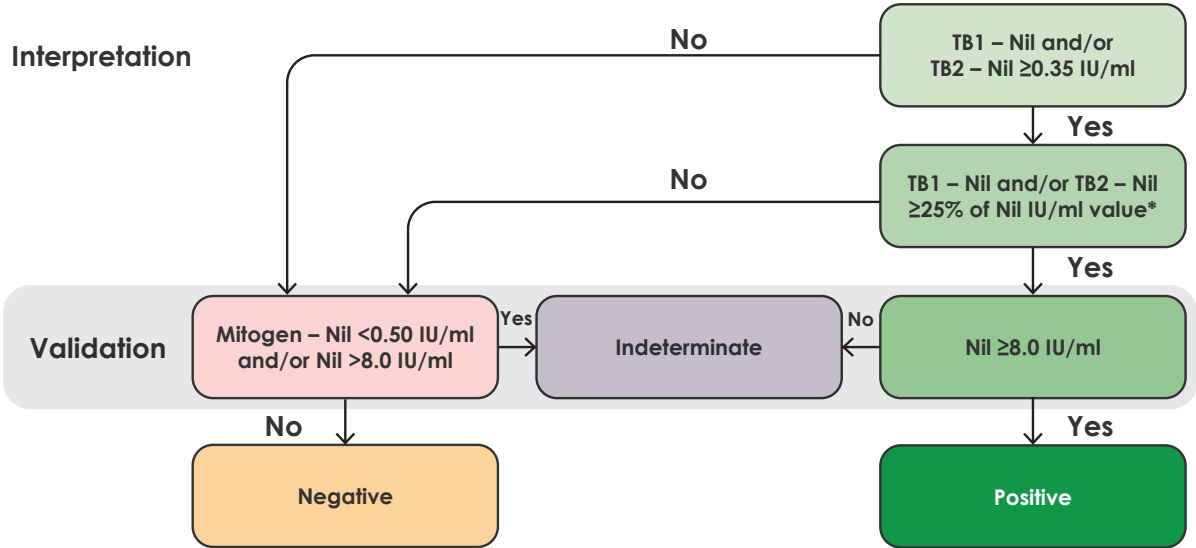
IU: international units.

Note: for TB1 minus nil or TB2 minus nil value to be valid, the ≥25% of the nil IU/mL value must be from the same tube as the original ≥0.35 IU/mL result.

Source: product insert *(1)*.

A2.2 WANTAI TB-IGRA – step by step

The procedures for collecting blood samples are similar to those of the QFT-Plus, except that direct blood draw into the incubation tubes is not recommended. Instead, blood samples are drawn into lithium heparin tubes and then transferred into the N, T and P tubes. One millilitre of whole blood sample is transferred into each of the test tubes. These tubes are then placed directly in a 37 °C ±1 °C incubator within 2 hours, and incubated for 20–24 hours. These steps are detailed below *(3)* and shown in Fig. A2.2.1 (up to incubation) and Fig. A2.2.2 (after incubation).

Step 1. Specimen collection

- Each tube should be labelled appropriately.
- For each patient, whole blood should be collected by venepuncture into a lithium heparin tube, and the volume collected should be at least 4 mL. The tube should be shaken well; it can then be stored at 20–27 °C for up to 16 hours before the specimen dispensing step.

Step 2. Specimen dispensing

- Each tube should be labelled appropriately.
- The lithium heparin tube should be gently inverted three to five times to mix the specimens.
- Next, 1 mL of the whole blood specimen should be dispensed into each of the N, T and P tubes.
- The N, T and P tubes should be gently inverted three to five times to mix the specimens.
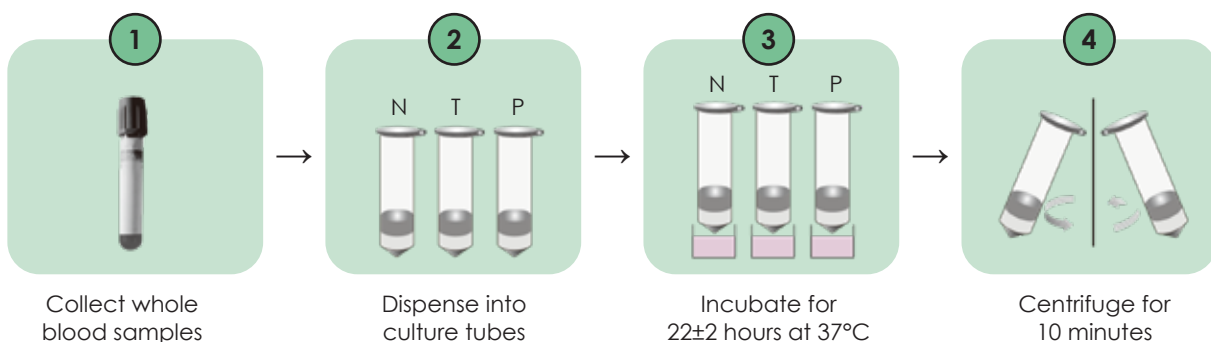
Step 3. Incubation

- Immediately after mixing, the N, T and P tubes should be placed in a 37 °C incubator.
- The tubes should be incubated in an upright position for 22±2 hours.

Step 4. Centrifugation

After incubation, all tubes should be centrifuged for 10 minutes at 3000–5000 rpm, then the supernatant should be aspirated.

Fig. A2.2.1. WANTAI TB-IGRA workflow – sample preparation and incubation

| Collect whole blood samples | Dispense into culture tubes | Incubate for 22±2 hours at 37°C | Centrifuge for 10 minutes |

Source: product insert *(3)*.

Step 5. ELISA

For the ELISA, 20 mL of a sample dilution buffer are added to microplate wells except the Blank well. Add 50 mL of the sample (supernatant), plus 50 mL of the standards solution into the respective wells except the Blank well, mix by tapping the plate gently and, then incubated for 60 minutes, after which horseradish peroxidase reagent is added. All wells are then incubated for a further 60 minutes. Following this, the wells are washed at least five times, ideally using an automated plate washer. Finally, colour A and colour B are added, then all wells are incubated for a further 15 minutes. A final reagent is added to stop the colorimetric action and then the optical density is read using a microplate ELISA reader.

Fig. A2.2.2. WANTAI TB-IGRA workflow – testing procedures after incubation
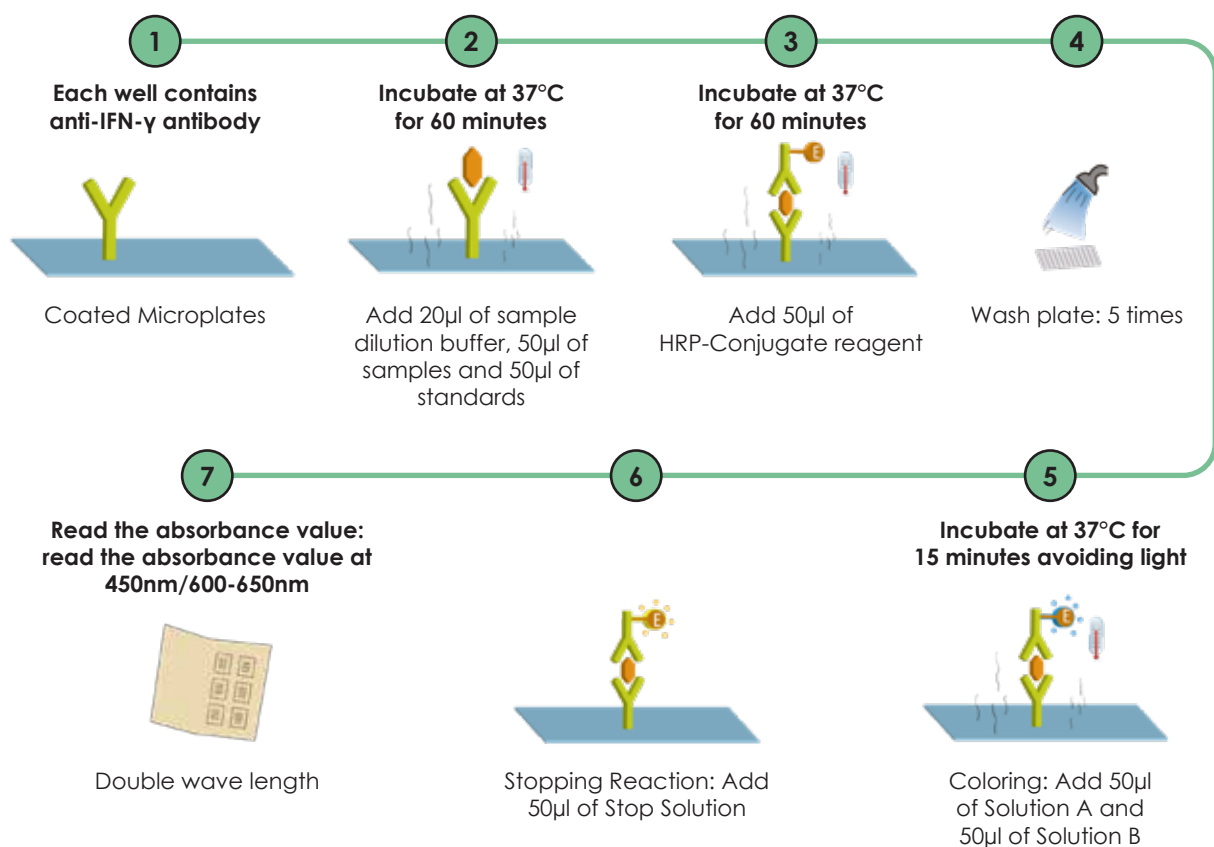
HRP: horseradish peroxidase; IFN-γ: interferon-gamma.

Source: product insert *(3)*.

Materials and supplies provided by manufacturer

The manufacturer provides the ELISA plate, testing reagents (N, T and P tubes) and standards. The components of the kit should be stored in a refrigerator at 2–8 °C. To assure maximum performance of this TB-IGRA kit, the reagents should be protected from contamination with microorganisms or chemicals during storage. Other materials required but not provided are listed below.

Equipment needed in the laboratory

The following equipment is required in the laboratory for the WANTAI TB-IGRA:

- dry incubator or water bath, 37 °C +0.5 °C;
- micro shaker for dissolving and mixing conjugate with samples;
- microwell plate reader, single wavelength 450 nm or dual wavelength 450 nm and 630 nm;
- microwell aspiration and wash system;
- timer; and
- appropriate waste containers for potentially contaminated materials.

Materials needed in the laboratory

The following materials are required in the laboratory for the WANTAI TB-IGRA:

- freshly distilled or deionized water;
- disposable gloves;
- disposable V-shaped troughs;
- dispensing system or pipette (single or multichannel) and disposable pipette tips; and
- absorbent tissue or clean towel.

Personnel needed

For the blood draw, a phlebotomist is required; this can be a nurse, laboratory technician, physician or other allied health professional.

In the laboratory, the steps following incubation are similar to those of the QFT-Plus, although they may be somewhat more labour-intensive. Substantial staff time is required for the centrifugation, pipetting, and mixing of reagents and samples; second incubation; further handling, washing and mixing; third incubation; and, finally, the ELISA measuring, which also involves setting up a standard curve.

Training, supervision and quality control

The personnel involved in this assay require initial training, and ongoing monitoring and evaluation for quality control (QC). The accuracy of the test results depends on the creation of an accurate standard curve. Therefore, the results from the standards must be examined before test sample results can be interpreted.

Precautions

Laboratory technicians should always wear protective clothing (including a laboratory coat, disposable gloves and eye goggles) when working with the chemical reagents needed for this test (3). The WANTAI TB-IGRA is intended for in vitro use only. Failure to adhere to the manufacturer's instructions may lead to an incorrect interpretation of the results.

Interpretation of WANTAI TB-IGRA

Interpretation of the test result is based on calibrating the standard curve using a series of diluted interferon-gamma standard solutions, then plotting the values for the positive, negative and TB antigen wells on a standard curve. The manufacturer recommends using the criteria shown in Table A2.2.1 when interpreting the WANTAI TB-IGRA results (3).

Table A2.2.1. Interpretation of WANTAI TB-IGRA test results

b	a-b	c-b	Interpretation
≤400	≥14 and >25% of b	Any value	Positive
	<14	≥20	Negative
	≥14 but <25% of b	≥20	
	<14	<20	Indeterminate
	>14 but <25% of b	<20	
>400	Any value	Any value	

a = The concentration of testing culture tube (T), b = the concentration of background control tube (N), c = the concentration of positive control tube (P).

Source: product insert *(3)*.

A2.3 T-SPOT.TB – step by step

The T-SPOT.TB has multiple steps *(4)*. These steps are detailed below and shown in Fig. A2.3.1 (for sample preparation and testing procedures) and in Fig. A2.3.2 (for interpretation).

Step 1. Blood-sampling procedures

The procedures for sample collection and preparation for T-SPOT.TB are different from those for QFT-Plus and WANTAI TB-IGRA. Whole blood samples should be collected into blood collection tubes (e.g. Vacutainer® CPT™ or lithium heparin tubes) and the tubes shaken to allow mixing of the heparin with the blood. The collected blood should be stored at room temperature (18–25 °C), and not refrigerated or frozen; about 8 mL of blood is needed. Next, working buffer and antibodies are added, which will bind T-cells (15-minute incubation at room temperature). Then, a bead reagent is added, which mediates the formation of antibody-mediated bead immune complexes (a further 15-minute incubation at room temperature).

Step 2. Peripheral blood mononuclear cell separation

White blood cells or peripheral blood mononuclear cells (PBMCs) should be separated. The procedure of separation of PBMCs from the collected blood is slightly different if using CPT blood collection tubes or lithium heparin tubes (check the manufacturer's instruction). Methods include centrifugation or the use of Ficoll® plates. Once separated, the PBMCs are washed several times, centrifuged and resuspended to eliminate all other products (e.g. gammaglobulins) that are normally in human blood and could interfere with the test. Next, PBMCs are counted because 250 000 cells (±50 000) are needed for each well of the test cells.

Step 3. Pre-coated well

After the PBMCs have been separated from the whole blood specimen, a standard number of PBMCs are added to four pre-coated wells supplied by the manufacturer: one containing nil (negative control), one containing phytohemagglutinin (positive control) and two containing *M. tuberculosis*–specific antigens. There is no need for a standard curve with this test.

Step 4. Incubation overnight

Enzyme-linked immunosorbent spot (ELISPOT) substrate is added to each well, along with 250 000 PBMCs. These wells are then incubated at 37 °C; the incubator needs to have 5% CO_2 and humidification.

Step 5. Wash, develop and dry plate

After the incubation, phosphate buffered saline (PBS) solution is used to wash the plate and is then discarded.

This washing should be repeated four times. After the fourth wash, conjugate reagent is added and incubated for 1 hour. The conjugate is then discarded and washing with PBS solution four times is repeated. Substrates are added and incubated for 7 minutes, then washing with PBS is again repeated four times. Finally, the plate is left to dry for 4 hours at 37 °C or 16 hours at room temperature.

Step 6. Count spots and interpret test results

Fig. A2.3.1. T-SPOT.TB workflow – sample preparation and testing procedures
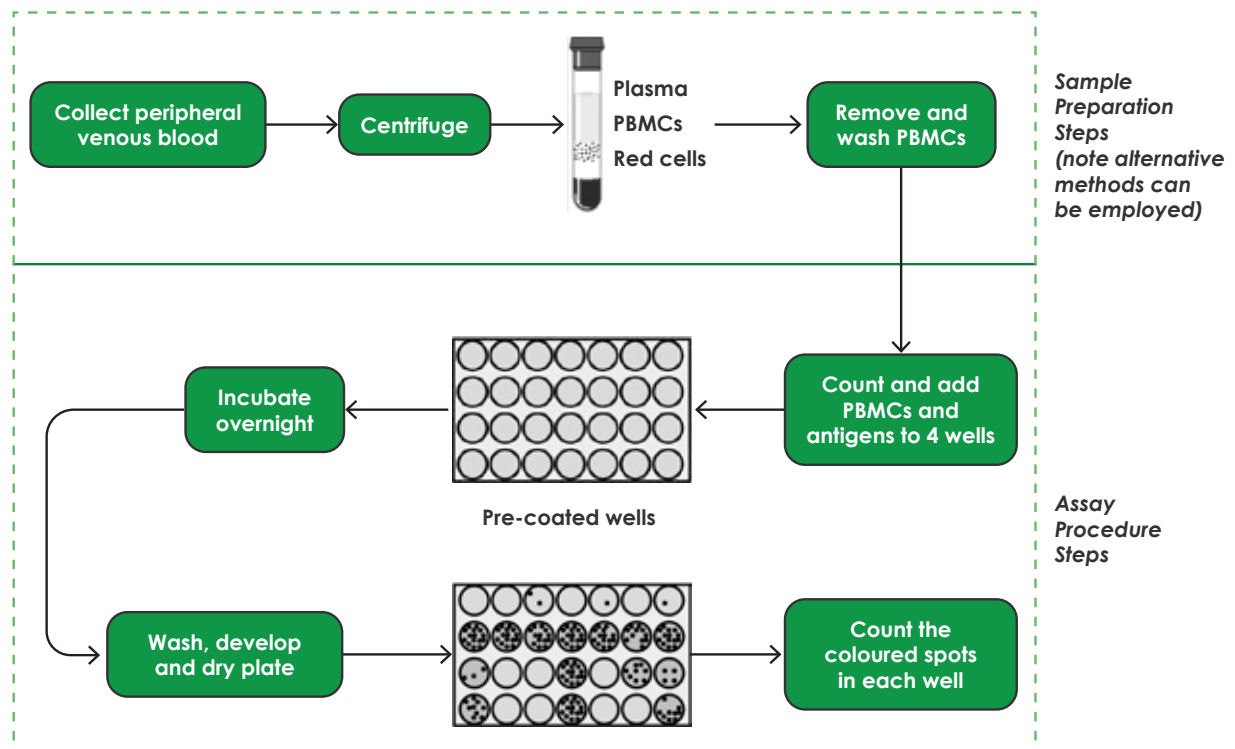
PBMC: peripheral blood mononuclear cell.

Source: product insert *(4)*.

Materials supplied by manufacturer

Materials provided by the manufacturer comprise a microtiter plate, panel A (which comprises early secretory antigenic 6 kDa [ESAT-6] antigens), panel B (which comprises culture filtrate protein 10 [CFP10] antigens) and a positive control (which contains phytohemagglutinin, conjugate reagent and substrate solution). These components of the kit should be stored at 2–8 °C, and prolonged exposure of the substrate solution to light should be avoided.

Equipment needed in the laboratory

The following equipment is required in the laboratory for the T-SPOT.TB:

– biohazard level 2 cabinet (recommended for reagent and sample preparation);
– timer;
– equipment to count the PBMCs (ideally, an automated counter such as a haematology analyser or dedicated cell counter);
– humidified incubator capable of maintaining a temperature of 37 °C, ±1 °C with 5% CO_2);
– microtiter plate washer or equipment to manually wash plates; and
– equipment for reading the spots on the plates (e.g. a microscope, digital microscope, magnifying glass or plate imager).

Materials needed

The following materials are required for the T-SPOT.TB:

– blood collection tubes (e.g. Vacutainer CPT or lithium heparin tubes);
– pipettes and sterile pipette tips; and
– reagents needed to count the PBMCs.

Personnel needed

A trained phlebotomist is required initially to draw blood samples and handle the samples. Laboratory technician time is needed for initial preparation (i.e. for the PBMC separation and counting, and setting up the wells), then significant technician time is needed after incubation for the washing and addition of reagents; finally, technician time is needed for counting the spots.

Training, supervision and QC

Personnel should be trained in the assay procedure and need to understand the instructions for use before performing the assay. Personnel require ongoing monitoring and evaluation for QC.

Precautions

Laboratory technicians should always wear protective clothing (including a laboratory coat, disposable gloves and eye goggles) when working with the chemical reagents needed for this test. The T-SPOT.TB is intended for in vitro use only. Failure to adhere to the manufacturer's instructions may lead to incorrect results.

Interpretation of T-SPOT.TB

Fig. A2.3.2 shows a flow diagram for the algorithm for interpretation of the results of the T-SPOT.TB.

Fig. A2.3.2. Algorithm flow diagram for interpretation of T-SPOT.TB
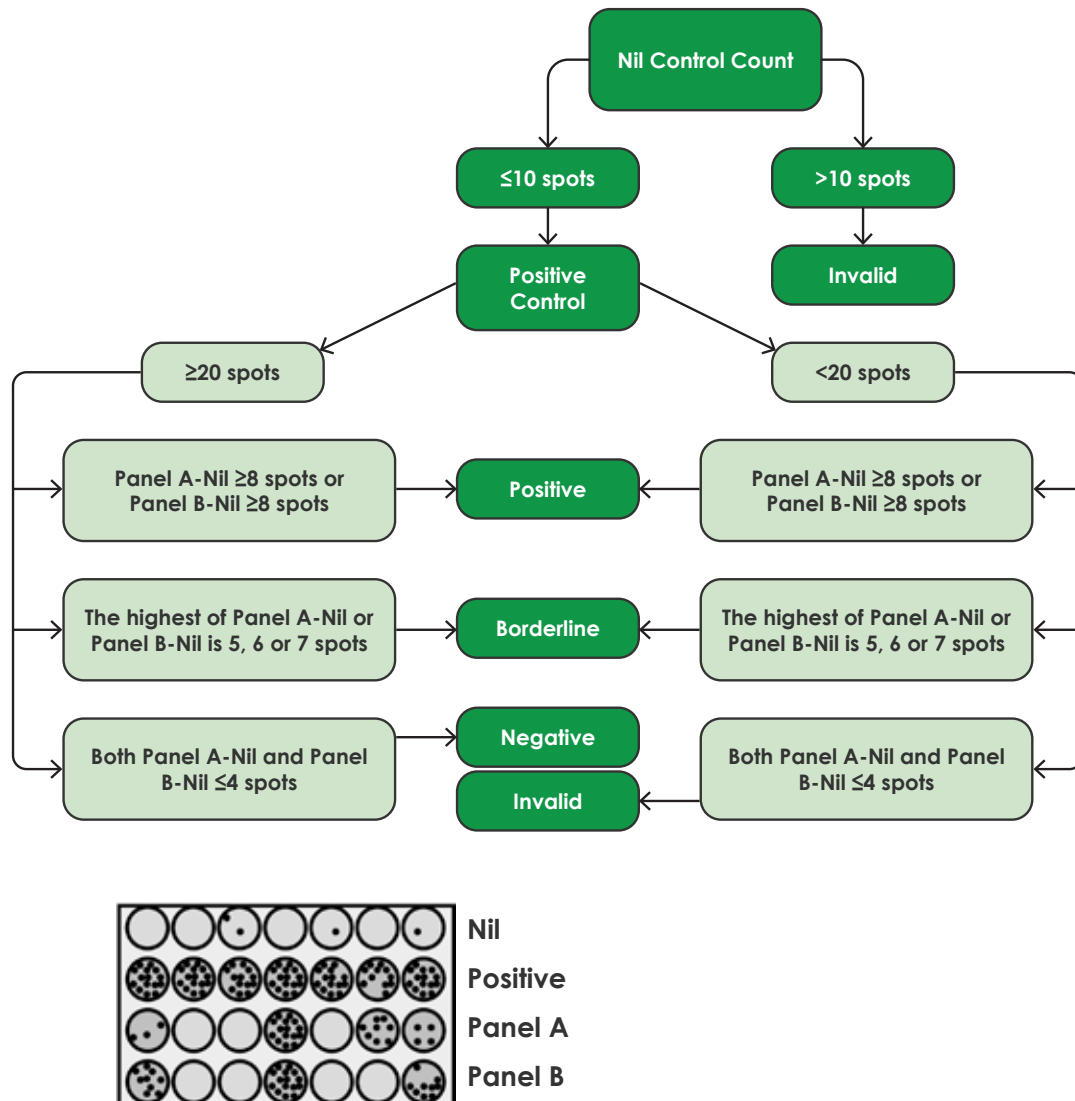
Source: product insert *(4)*.

Results for the T-SPOT.TB test are interpreted by subtracting the spot count in the nil control well from the spot count in each of the panels, according to the following algorithm provided by the manufacturer *(4)*:

- The test result is *positive* if (Panel A-Nil) and/or (Panel B-Nil) ≥8 spots (Table A2.3.1, below).
- The test result is *negative* if both (Panel A-Nil) and (Panel B-Nil) ≤4 spots (includes values of zero) (Table A2.3.2, below).

- The test result should be considered borderline if the highest of the spot counts from Panel A or Panel B is such that the (Panel minus Nil) spot count is 5, 6 or 7 spots. In such cases, re-testing by collecting another patient specimen is recommended (Table A2.3.3, below).
- If the test result is still borderline, then other diagnostic tests or epidemiological information should be used to help determine the patient's TB infection status.

T-cells releasing interferon-gamma are counted using an ELISPOT assay. The test result is reported as the number of interferon-gamma producing T-cells (i.e. spot-forming cells), and the result is classified by the manufacturer as *positive*, *negative* or *borderline/equivocal*.

Table A2.3.1. Positive T-SPOT.TB interpretation: either (Panel A-Nil) or (Panel B-Nil) ≥8 spots

Nil control well count	Either Panel A or Panel B has the following number of spots[a]	Result interpretation
0	≥8	Positive
1	≥9	Positive
2	≥10	Positive
3	≥11	Positive
4	≥12	Positive
5	≥13	Positive
6	≥14	Positive
7	≥15	Positive
8	≥16	Positive
9	≥17	Positive
10	≥18	Positive
>10 spots	n/a	Invalid[b]

[a] The highest Panel-Nil spot count is to be used to determine the test outcome.

[b] In the case of invalid results, these should be reported as "Invalid" and it is recommended to collect another sample and re-test the individual.

Table A2.3.2. Negative interpretation: both (Panel A-Nil) or (Panel B-Nil) ≤4 spots

Nil control well count	Both Panel A or Panel B have the following number of spots	Result interpretation
0	≤4	Negative
1	≤5	Negative
2	≤6	Negative
3	≤7	Negative
4	≤8	Negative
5	≤9	Negative
6	≤10	Negative
7	≤11	Negative
8	≤12	Negative
9	≤13	Negative
10	≤14	Negative
>10 spots	n/a	Invalid[a]

[a] In the case of invalid results, these should be reported as "Invalid" and it is recommended to collect another sample and re-test the individual.

Table A2.3.3. Borderline (equivocal) interpretation: the highest of (Panel A-Nil) or (Panel B-Nil) is 5, 6 or 7 spots

Nil control well count	The highest of Panel A or Panel B has the following number of spots	Result interpretation
0	5, 6 or 7	Borderline (equivocal)[a]
1	6, 7 or 8	Borderline (equivocal)[a]
2	7, 8 or 9	Borderline (equivocal)[a]
3	8, 9 or 10	Borderline (equivocal)[a]
4	9, 10 or 11	Borderline (equivocal)[a]
5	10, 11 or 12	Borderline (equivocal)[a]
6	11, 12 or 13	Borderline (equivocal)[a]
7	12, 13 or 14	Borderline (equivocal)[a]
8	13, 14 or 15	Borderline (equivocal)[a]
9	14, 15 or 16	Borderline (equivocal)[a]
10	15, 16 or 17	Borderline (equivocal)[a]
>10 spots	n/a	Invalid[b]

[a] Results where the highest of the Panel A or Panel B spot count is such that the (Panel minus Nil) spot count is 5, 6 or 7 spots should be considered borderline (equivocal) and re-testing by collecting another patient specimen is recommended.

[b] In the case of invalid results, these should be reported as "Invalid" and it is recommended to collect another sample and re-test the individual.

References for Annex 2

1. QuantiFERON®-TB Gold Plus (QFT®-Plus) ELISA package insert. Germany: QIAGEN; 2019 (http://www.quantiferon.com/wp-content/uploads/2017/04/English_QFTPlus_ELISA_R04_022016.pdf).

2. Campbell J, Pease C, Daley P, Pai M, Menzies D. Diagnosis of tuberculosis infection. In: Menzies D (ed), Canadian tuberculosis standards – 8th edition. Ottawa: Canadian Thoracic Society; 2022:49–65 (https://www.tandfonline.com/doi/full/10.1080/24745332.2022.2036503).

3. WANTAI TB-IGRA package insert. Beijing: Beijing Wantai Biological Pharmacy Enterprise Co., Ltd; 2022.

4. T-SPOT.TB package insert. United Kingdom: Oxford Immunotec; 2017 (http://www.oxfordimmunotec.com/international/wp-content/uploads/sites/3/Final-File-PI-TB-US-V6.pdf).

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