2. Methods of Tuberculosis Screening

Diagnosis of active or latent TB involves a number of tests. There is no gold standard for determining whether a person is infected with *M. tuberculosis* but in practice the tuberculin skin test (TST) is the standard method used. Recently immunological blood tests i.e. interferon-gamma release assays (IGRA) have been developed to aid diagnosis. However, neither the TST nor IGRA can be used to distinguish latent infection from active disease.

2.1 Definition of Active TB

Active TB is defined as infection with mycobacteria of the *M. tuberculosis* complex, where mycobacteria are growing and causing symptoms and signs of disease. This is distinct from LTBI where mycobacteria are present but are inactive and not causing symptoms of disease. The diagnosis of active TB is made most often on the basis of positive bacteriology but in approximately 15%-25% of cases on the basis of appropriate clinical and/or radiological and/or pathological presentation as well as treatment response (see chapters 4 and 5).

2.2 Definition of Latent TB Infection

A person with LTBI usually has a positive TST or IGRA test but has no physical findings of TB disease and the chest X-ray is normal or only reveals evidence of healed infection i.e. granulomas or calcification in the lung, hilar lymph nodes or both. Persons with LTBI are asymptomatic and are not infectious (see table 3.1, chapter 3).

The three screening methods discussed in this chapter are:

1. Tuberculin skin test
2. Interferon-Gamma Release Assays (IGRA)
3. Chest X-ray.

2.3 Tuberculin Skin Test

The main tool to diagnose TB infection is the TST. It is also used as an aid to the diagnosis of active TB disease. In Ireland, the Mantoux test is the TST used. This test consists of the intradermal injection of a small amount of purified protein derived from *Mycobacterium tuberculosis* bacteria (PPD). The local skin reaction to PPD is used to assess an individual's sensitivity to the tuberculin protein. In a person who has cell-mediated immunity to these tuberculin antigens, a cell-mediated delayed hypersensitivity reaction will occur within 48 to 72 hours. The reaction will cause localised swelling and will be manifest as induration of the skin at the injection site. The greater the reaction, the more likely it is that an individual is infected or has active TB disease. In persons who are newly exposed and become infected with TB, this cell-mediated reaction to tuberculin will develop 3 to 8 weeks later.[28]

WHO recommends that the Mantoux 2TU/0.1ml tuberculin PPD should be used and this is the standard test recommended for use in Ireland. A comparative study undertaken by Comstock *et al* revealed that 2TU has almost identical sensitivity to the use of 5TU for tuberculin testing although specificity was slightly lower with 2TU.[29] However, it was concluded that the results of testing with 2TU and 5TU were sufficiently similar to deem them biologically equivalent.[29]

Recommendation:

The standard tuberculin skin test (TST) recommended for use in Ireland is the Mantoux 2TU/0.1ml tuberculin PPD. Mantoux 10TU/0.1ml tuberculin PPD is not recommended for use in Ireland.

In general testing for LTBI is indicated when the risk of development of disease is increased if the patient is infected.

There are three general situations when the disease risk is increased:

- **Recent infection**: most commonly contacts of a patient with a recent diagnosis of infectious TB disease ***or*** immigrants or persons from countries of high TB incidence (≥40 per 100,000 TB cases notified per year) within 2 years of arrival in Ireland
- **Increased risk of reactivation due to impaired immunity**: This includes HIV infection and immunosuppressed conditions e.g. diabetes, renal failure, immunosuppressant medications and pulmonary silicosis
- **When there is radiological evidence of old, healed inactive TB but no prior treatment.**[30]

The following persons should not receive a TST:

- Those who had severe blistering TST reactions in the past or with extensive burns or eczema present over TST testing sites, because of the greater likelihood of adverse or severe reactions
- Those with documented active TB disease or a well-documented history of adequate treatment for TB infection or disease in the past. In such patients the test is of no clinical utility.
- Those with major viral infections e.g. varicella (chickenpox), measles, mumps, infectious mononucleosis ***BUT*** not the common cold or minor viral infections
- Those who received MMR vaccines in the previous four weeks as this has been shown to increase the likelihood of false negative TST results. No data are available in relation to the effect of other live virus vaccines e.g. varicella or yellow fever but it would be prudent to follow the same four week guidance.[30]

The following persons can receive a TST:

- Those with a common cold
- Those who are pregnant or breastfeeding
- Those immunised with any vaccine on the same day
- Those immunised within the previous 4 weeks with inactivated vaccines
- Those who give a history of a positive TST reaction (other than blistering) that is not documented
- Those taking low doses of systemic corticosteroids, < 15mg prednisolone (or equivalent) daily. It generally takes a steroid dose equivalent to ≥15mg prednisolone daily for 2-4 weeks to suppress tuberculin reactivity.[30;31]

Administration of the Mantoux test

In all cases, the Mantoux test should be administered intradermally. This is also sometimes referred to as intracutaneous administration. The Mantoux test is normally performed on the flexor surface of the left forearm at the junction of the upper third and the lower two-thirds. If the skin is visibly dirty, it should be washed with soap and water. The Mantoux test is performed using a 0.1ml tuberculin syringe or alternatively a 1ml graduated syringe fitted with a short bevel 26G (0.45x10mm) needle. A separate syringe and needle must be used for each subject to prevent cross-infection. Then 0.1ml of PPD should be drawn into the tuberculin syringe and the 25G or 26G short-bevelled needle attached to give the injection. The needle should be firmly attached and the intradermal injection administered with the bevel facing uppermost.

The operator stretches the skin between the thumb and forefinger of one hand and with the other hand slowly inserts the needle with the bevel upwards for about 5mm into the superficial layers of the dermis almost parallel to the surface. The needle can usually be seen through the epidermis. A correctly given intradermal injection results in a tense blanched raised bleb and considerable resistance is felt when the fluid is being injected. A bleb is typically of 7mm diameter following a 0.1ml intradermal injection. If little resistance is felt when injecting and a diffuse swelling occurs rather than a tense bleb the needle is too deep and should be withdrawn and reinserted intradermally on the opposite forearm or on the same forearm at a site at least 10cm away from the previous injection. Do not cover the site with a bandage. Inform the patient that he or she should not scratch the site but may perform all normal activities including showering or bathing. Record the following details: a) date of injection; b) dose (2TU, 0.1ml); c) manufacturer; d) lot number; e) expiry date; f) site of injection and g) person who administered the injection.

Detailed instructions on performing a Mantoux test are available on the National Immunisation Office website at www.immunisation.ie/en/Downloads/PDFFile_14983_en.pdf.

Similar instructions are also available on the Staten Serum Institut (Denmark) website at www.ssi.dk/sw11710.asp.

Recommendation:
In all cases, the TST (Mantoux test) should be administered intradermally.

Storage

Care should be taken to store PPD Mantoux tests and BCG vaccine in separate areas of the fridge to ensure the correct product is administered. If using the vial of Tuberculin PPD (Mantoux) on more than one patient, it is recommended that once the vial is in use, it should be used immediately. Otherwise it should not be in use for longer than 24 hours and stored between 2 and 8°C as per Tuberculin PPD product specific details from Statens Serum Institut (SSI) in Copenhagen. [32] In light of the need for the immediate use of the vial of PPD as indicated above, it is recommended that where possible a clinic should be arranged to undertake Mantoux testing on more than one person.

Reading the TST (Mantoux test)

The TST should be read by a trained health professional. Individuals without experience in reading a TST may not feel slight induration and the result may be mistakenly recorded as 0mm.

TST interpretation depends on a number of factors as follows:

- Measurement of the induration in millimetres
- The person's risk of being infected with TB and of progression to disease if infected
- Prior BCG vaccination or exposure to non-tuberculous mycobacteria (NTM) (section 2.4)
- Conditions resulting in a false negative result (section 2.4).

The results should be read within 48 to 72 hours of receiving the test but a valid reading can usually be obtained up to 96 hours later. The transverse diameter of the area of induration and not the erythema at the injection site is measured with a ruler and the result recorded using millimetres. As several factors affect interpretation of the test, the size of the induration should be recorded and **NOT** just as a positive or negative result.

It is recommended that the following items are recorded:

- Date the induration was read
- Measurement of the induration if any in millimetres
- Any adverse reactions e.g. blistering (can occur in 3-4% of subjects) and
- The name of the individual who read the test.

There is some variability in the time at which the test develops its maximum response. The majority of tuberculin-sensitive subjects will be positive at the recommended time of reading. The predictive value can be enhanced by using cut-off points dependent on the infection risk. The reaction to a TST is classified as positive based on the individual's risk factors (see table 2.1). In general, a negative TST result is ≤ 5mm. For the interpretation of TST results in contact tracing situations and in screening of HCWs and new entrants, see chapters 8 and 9.

Recommendation:

The TST (Mantoux test) result should be read within 48 to 72 hours of receiving the test. The transverse diameter of the area of induration (and not the erythema at the injection site) is measured with a ruler and the result recorded using millimetres.

Note:

- A delay in reading the TST if the result is positive i.e. >5mm does not affect the validity of the results
- A strongly positive TST resulting from inadvertent subcutaneous administration does not affect the validity of the reading.

Deciding that a TST is positive

The health professional reading the TST must decide whether the test result is positive. This is based on the size using the criteria listed in table 2.1. Once a TST is considered positive the individual should be referred for a medical evaluation. There is no clinical utility in performing a TST in the future once a test that was properly performed and read is considered positive.[30]

Medical evaluation

This should include assessment of symptoms suggestive of possible active TB, risk factors for TB such as contact history or other medical illness and a chest X-ray. In the event of symptoms or an abnormal chest X-ray, sputum for acid-fast bacteria smear and culture should be taken. In persons with no evidence of TB disease, treatment of LTBI should be considered.

Table 2.1: Categories of response to TSTs (Mantoux tests) based on individual's risk factor(s) for development of TB disease[33] ᵠ

An induration of >5mm is considered positive in:	• HIV-infected persons • A recent contact of a person with active TB disease • Persons with fibrotic changes on chest X-ray consistent with prior TB and no documented treatment • Persons with organ transplants and other immunosuppressed persons e.g. those taking the equivalent of ≥15mg/day of prednisolone for one month or longer or taking TNF-alpha antagonists • Children aged < 5 years (with no BCG) from a country with a high incidence of TB (≥ 40/100,000 per year)
An induration of ≥ 10mm is considered positive in:	• Immigrants: Persons (aged 16 to 35 years) who have immigrated within the past 5 years from countries with a very high TB incidence (>500/100,000) and children aged 5 to 15 years who have immigrated (within the past 5 years) from countries with TB incidence ≥40/100,000 per year • All children <5 years of age or children/adolescents exposed to adults in high-risk categories* • Injecting drug users • Residents or employees of high risk congregate settings e.g. prisons, homeless shelters • Mycobacterial laboratory personnel • Persons with clinical conditions which place them at increased risk of progression to active TB e.g. silicosis, diabetes mellitus • HCWs from high incidence countries (≥40 cases per 100,000 population per year)† ‡
An induration of ≥ 15mm is considered positive in:	• Irish HCWs and HCWs from countries where the annual rate of TB is <40 per 100,000§ • All others i.e. any person including persons with no known risk for TB. However, targeted skin testing programmes should only be conducted among high risk groups.

ᵠ In the context of serial tuberculin skin testing an increase of >5mm represents conversion and recent exposure.

* Recent converters, HIV-positive individuals, persons receiving immunosuppressive therapy i.e. tumour necrosis factor-α (TNF-α) antagonist, persons with evidence of old healed TB lesions on chest X-ray i.e. fibronodular disease/non-calcified fibrotic lesions (if not previously treated or if treated, and not adequately treated), foreign-born persons from countries with high TB endemnicity, homeless persons, IDUs and HCWs

† Such patients should generally be referred to a respiratory or infectious disease clinician for assessment. However, an occupational medicine consultant may wish to treat these patients if appropriate protocols, audit of care and resources are in place

‡ HCWs from countries of high TB incidence have a higher risk of having TB, hence the lower cut-off point for considering LTBI

§ Such patients should generally be referred to a respiratory or infectious disease clinician for assessment. However, an occupational medicine consultant may wish to treat these patients if appropriate protocols, audit of care and resources are in place

2.4 Factors Affecting the Result of the Tuberculin Skin Test

False positive TST results

Although for persons with LTBI and normal immune responses the test sensitivity approaches 100%,[34] false positive results can occur because of the sensitising effect on the immune system of either prior BCG vaccination or NTM.

BCG and NTM have important effects on the predictive value of the TST when the expected prevalence of true LTBI is low such as in Western Europe or North America. In contrast, when the expected prevalence of tuberculous infection is high, such as close contacts of a smear positive pulmonary case or persons from high TB incidence countries, then the predictive value of a positive TST result is high. A study by Menzies *et al* in 1992 showed that BCG leading to a false positive TST was more common among participants from low-incidence countries compared to those from countries of high endemnicity for TB.[35]

Menzies and Doherty[36] state that although all recipients of BCG will have positive tuberculin reactions within two months of vaccination with BCG, these reactions will wane over time. Studies by Menzies *et al* (1992, 1994) indicate that for those vaccinated with BCG in infancy, only 3-5% manifest a positive TST when tested 5 years after the vaccination.[37;38] This may reflect the relative immaturity of the immune system in infants although protective efficacy if anything is higher.[39;40] Of those vaccinated at an older age, tuberculin reactions are larger and wane more slowly. In this older cohort, on average 30-35% will have BCG-related positive TST results even after an interval of more than 10 to 15 years.[37-39;41-43] Post-BCG vaccination can account for up to 10mm of induration, and there is no published evidence to suggest that this sensitivity correlates with immunological protection.[44-47]

Note:

Care should be taken when attributing BCG vaccination as a cause of a positive TST if:[30]

- BCG vaccine was given in infancy and the person tested is now aged 10 years or older
- There is a high probability of TB infection i.e. close contacts of an infectious TB case or immigrants (including HCWs) from countries with high annual TB incidence (see table 2.1)
- There is a high risk of progression from TB infection to disease (see table 3.2)
- Any TST ≥15mm induration should not be attributed to BCG vaccination.

False negative TST results

The reaction to tuberculin protein may be suppressed by the following:

- Cutaneous anergy (anergy is the inability to react to skin tests because of a weakened immune system)
- Recent TB infection (within eight to 10 weeks of exposure)
- Old TB infection
- Age*:* very young age (less than three months old) and the elderly
- Major viral infections e.g. varicella (chickenpox), measles, mumps, infectious mononucleosis
- Recent live viral vaccination e.g. measles, mumps, rubella, varicella and yellow fever (tuberculin testing should not be undertaken within four weeks of having received a live viral vaccine)
- Malnutrition, particularly when there has been recent weight loss[48]
- Extensive TB disease (pulmonary or miliary) can itself also temporarily depress immunity and can lead to a paradoxically negative TST[49]
- Other illnesses e.g. malignancies especially lymphoma, renal failure, sarcoidosis, diabetes mellitus
- Immunosuppression due to disease including HIV infection
- Immunosuppression due to treatment including cytotoxics, corticosteroid therapy (≥15mg prednisolone daily for four weeks or longer), transplant therapy and infliximab
- Incorrect method of TST administration.[50] This should be avoidable.
- Incorrect interpretation of the TST reaction
- Insufficient dose of PPD
- Inactive tuberculin PPD: tuberculin PPD vials must be used within 24 hours of opening.

Subjects who have a negative test but who may have had one of the major viral infections (excluding the common cold) outlined above at the time of testing or at the time of reading the test should be re-tested two to three weeks after clinical recovery before being given BCG.

2.5 Conversion and Boosting

A newly positive TST after an initial negative test result and an increase of > 5mm represents a true biological phenomenon in two consecutively performed TSTs. This could be due to conversion or boosting.

Conversion

Conversion is defined as the development of new hypersensitivity to mycobacteria following exposure to new TB or NTM infection including BCG vaccination.[36] A conversion is presumptive evidence of new *M. tuberculosis* infection and poses an increased risk for progression to TB disease indicating a change from being uninfected to infected.[51]

Conversion is more likely in a previously tuberculin negative individual or in a situation of high risk of exposure to TB such as in a close contact of a sputum smear positive index case or in an outbreak investigation. If a person who has a documented negative TST result within the previous 12 months is exposed to an infectious TB case, then only one TST (Mantoux test) is necessary to detect conversion. Persons who demonstrate TST conversion should be investigated for active disease or LTBI.[36]

Boosting

Boosting is defined as the recall of non-specific immunity in the absence of new infection and is mainly seen in adults and older persons.[36] When non-specific or remote sensitivity to tuberculin (PPD in the skin test) wanes or disappears with time, subsequent tuberculin skin tests can restore the sensitivity.[51] An initially limited reaction size is followed by a larger reaction size on a later test, which can be confused with a conversion or a recent *M. tuberculosis* infection. If an increase in reaction size is noted after one to three weeks and there has been little or no possibility of exposure then it is likely that the increase is due to boosting.[51] Boosting is best distinguished from conversion on clinical grounds.

Two-step testing

Two-step testing is used to distinguish new infections from boosted reactions in infection control and prevention surveillance programmes. This method is not recommended for testing contacts of infectious TB cases. A contact whose second test result is positive after an initial negative result should be classified as recently infected.[51]

In persons who may be liable to boosting in whom it is important to establish a true baseline TST response, a second TST can be administered one to three weeks after the first. The second test should be done on the other arm; repeat testing at one site may alter the reactivity either by hypo- or more often hyper-sensitising the skin and a changed response may only reflect local changes in skin sensitivity. The result of the second boosted reaction is the correct result, that is the result which should be used for decision making and future comparison. This two-step approach can reduce the likelihood that a boosted reaction to a subsequent TST will be misinterpreted as recent infection.

Two-step testing is indicated in the following situations when the first TST (Mantoux test) in the two-step series is negative:

(a) Where serial tuberculin tests are to be used as in HCWs; and

(b) When tuberculin testing those with previous BCG vaccination, *this does not apply to contacts of infectious cases who will already have been re-sensitised when transmission has occurred.* [33;52]

If the second test is positive (table 2.1), it is recommended that the individual is referred for medical evaluation including a chest X-ray and they should not undergo further tuberculin testing. If the chest X-ray is normal and there are no associated factors that increase the risk of TB reactivation, then preventive therapy is not indicated.

The two-step test needs to be performed **once** only if properly performed and documented. It never needs to be repeated. Any subsequent TST can be one step regardless of how long it has been since the last TST.[30]

2.6 Interferon-Gamma Release Assays (IGRA)

In recent years in-vitro immunological assays called IGRA have been developed to diagnose TB infection. These assays involve a single blood test and operate on the basis that T-cells which have been in previous contact with TB antigens release high levels of the cytokine interferon gamma when they are re-exposed to the same mycobacterial antigens.[53] This reaction is specific to a small number of mycobacteria including *M. tuberculosis* but not to the BCG vaccine strain of *M. bovis* and consequently are less influenced by prior BCG vaccination than TSTs. The amount of interferon gamma or the number of *M. tuberculosis* sensitive T-cells in the blood is then estimated by the tests. T-cells which have not been in contact with the bacterium will not release cytokine.[54]

There are currently two IGRA assays commercially available for use: QuantiFERON-TB Gold® In –Tube (Cellestis Ltd., Australia) and T-SPOT.TB (Oxford Immunotec) (see chapter 4).

QuantiFERON-TB Gold® In –Tube assay measures the release of interferon gamma in whole blood in response to stimulation by ESAT 6 and CFP 10. In the T-SPOT.TB test, individual activated ESAT 6, CFP 10 and TB 7.7 specific T-cells are enumerated using the ELISPOT methodology. It has been suggested that the T-SPOT.TB test may be more sensitive than QuantiFERON-TB Gold® In –Tube in children under five years of age and in immunocompromised patients.[55]

International guidelines on the use of IGRA

In 2005, CDC recommended that the Food and Drug Administration (FDA)-approved version of QuantiFERON-TB Gold® In –Tube assay may be used in place of the TST for all indications including contact tracing and serial testing of healthcare workers.[56;57] In 2007, an updated version QuantiFERON-TB Gold® In –Tube was approved for this function. In 2006, the UK National Institute for Health and Clinical Excellence (NICE) guidelines recommended a hybrid two-step approach for LTBI diagnosis; initial screening with TST and subsequent IGRA testing (if available) for those who are TST positive (or in whom TST may be unreliable) to confirm TST results.[26]

Evidence from international studies suggests that IGRA have a higher specificity than tuberculin skin tests and have less potential for false positive results.[26;58;59] Both IGRA are very specific (93% to 99%) and are unaffected by prior BCG. While several QuantiFeron studies have consistently shown very high specificity, data are limited on the specificity of the commercial T-SPOT.TB assay.[60] A number of systematic reviews have been published which support this view.[54] The results of a meta-analysis carried out in 2007 however, is not so clear cut. While both IGRA tests were more specific than TSTs when applied to all patients, the difference between IGRA and TST disappeared when patients known to have BCG vaccine were excluded. Both IGRA tests were more sensitive than TST with the T-SPOT.TB assay displaying higher sensitivity (~90%) compared to the QuantiFERON-TB Gold® (approximately 75% to 80%).[60 61]

Use of IGRA also showed little evidence of being affected by prior BCG vaccination and stronger correlation with exposure categories than did tuberculin skin tests. This was shown in low prevalence groups, in household contacts and in outbreak situations.[62;63] A recent study on the use of TST versus IGRA for the diagnosis of LTBI in a HCW population in Germany concluded that TST overestimates the prevalence of LTBI in HCWs and recommended that a positive TST result should be verified by IGRA. However, they also concluded that more studies are required in order to confirm that IGRA are more sensitive in diagnosing LTBI than the TST.[64] In conclusion, from the evidence, it is currently justifiable to assume that IGRA are at least as sensitive as TST and more specific in populations that include previously BCG vaccinated individuals.

The results of a limited number of studies, published to date, assessing the predictive value of IGRA are inconclusive. A recent German study found that 14.6% of IGRA positive individuals developed active TB compared to 5.6% of TST positive contacts.[65]

Recommendations for the use of IGRA

IGRA use should be considered in conjunction with a clinical and public health risk assessment. If available, IGRA can be used for the diagnosis of LTBI in the following settings.

Contact tracing (see chapter 8)

- The TST (Mantoux test) should be used as the first line test for the diagnosis of LTBI in contacts of infectious TB cases and others considered to be at high risk of LTBI. Those with positive TST results should be considered for IGRA testing, if available (see figures 8.1 & 8.2)
- IGRA may be considered on a case by case basis in adults and children as per the general recommendations in section 8.7.[60]

Pre-placement screening of HCWs

In new HCWs who are asymptomatic for TB and have a low pre-test probability of LTBI, IGRA, if available, can be used to confirm a positive TST result. Persons with a positive IGRA should be considered for treatment of LTBI (see chapter 9).

New entrant screening

Although the use of IGRA in screening new entrants has not clearly been demonstrated to date, the use of IGRA can be considered:

- As a confirmatory test in those individuals with a positive TST
- In screening new entrants with concomitant conditions that increase the individual's risk of reactivation of LTBI (chapter 9).

For individuals commencing on immunosuppressive therapy, i.e. tumour necrosis factor-α (TNF-α) antagonists.

- IGRA, if available, can be used as an adjunct to screening in addition to a medical history, chest X-ray and TST.

IGRA, if available, can be considered as the sole test for LTBI in the situation outlined below:

- When screening large numbers of individuals as part of a public health investigation where logistic issues make repeated visits for sequential testing impractical.[54]

Recommendation:

For contact tracing, the TST (Mantoux test) should be used as the first line test for the diagnosis of LTBI in contacts of infectious TB cases and others considered to be at high risk of LTBI. Those with positive TST results should be considered for IGRA testing (see figures 8.1 & 8.2). IGRA may be considered on a case by case basis in adults and children as per the general recommendations in section 8.7.[60]

IGRA performance in immunocompromised populations

There are few studies on the sensitivity and specificity of IGRA in immunocompromised populations. TST sensitivity is modest to poor in these populations. The sensitivity of T-SPOT.TB appears to be maintained in immunocompromised individuals and appears to have a higher rate of positivity than TST. QuantiFeron studies have not demonstrated this.[60] In the immunocompromised person (adult or child), the TST should be the initial test used to detect LTBI. If the TST is positive, the person should be considered to have LTBI. However, in light of the known problem of false negative TST results in immunocompromised populations, a clinician still concerned about the possibility of LTBI in an immunocompromised person with an initial negative TST result may perform an IGRA test. If the IGRA test is positive, the person might be considered to have LTBI. If the IGRA result is indeterminate, the test should be repeated to rule out laboratory error. If the second test is negative, the clinician should suspect anergy and rely on the person's history, clinical

features and other laboratory results to make a decision on the likelihood of LTBI. Either IGRA test may be used, however, there is evidence that the T-SPOT.TB assay may be more sensitive that the QuantiFeron test and this will be especially relevant for immunocompromised populations.[60]

While the approach of accepting either test result (TST or IGRA) as positive will improve the sensitivity of detecting LTBI in immunocompromised populations, there are no data supporting the efficacy of preventive therapy in TST negative but IGRA positive individuals. Thus the clinician must weigh the potential benefit of detecting more persons with positive test results against the lack of evidence for the benefit of preventive therapy in such persons.

Potential boosting of IGRA by previous TST

Previous guidelines by CDC state that the results of IGRA are not influenced by previous TST.[51] Studies by Leyten *et al*[66] and Richeldi *et al*[67] report no boosting phenomenon following the evaluation of T-SPOT. TB assay. Some studies however, have reported boosting of the QuantiFERON-TB Gold® In –Tube assay results when taken 6 to 8 weeks after a TST.[68;69] Although these studies have limitations, there are animal studies suggesting that TST might boost subsequent measurements of interferon gamma.[70;71] This issue requires further investigation. Due to these concerns, it is recommended by both the HPA and the Public Health Agency of Canada that the IGRA test should be undertaken at the time of reading the Mantoux results.[54;60]

Serial testing

There are insufficient data to inform recommendations on serial testing with IGRA. Studies of serial testing of individuals with either LTBI or TB disease do not show a clear pattern. In some studies, IGRA responses increased,[72] decreased[73] or showed no change.[74] Further studies are needed.

Diagnosis of active TB disease

IGRA should not be used in the first instance for the diagnosis of active TB disease in either adults or children and should not replace the appropriate microbiological and molecular investigation.

Culture remains the gold standard for the diagnosis of TB disease as it provides a definitive diagnosis and permits the identification of drug resistance. IGRA have no benefits in known pulmonary TB cases with bacteriological/molecular confirmation.

Recommendation:
IGRA tests should not be used in the first instance for the diagnosis of active TB disease. Appropriate microbiological and molecular investigations remain the gold standard.

However, in some patients (adults and children) with TB, it is not possible to isolate *M. tuberculosis* from clinical specimens or to obtain clinical specimens, despite the individual having symptoms, signs and/or radiological changes consistent with the diagnosis of TB. In these circumstances, a positive IGRA may increase confidence in the diagnosis. In those with symptoms or signs compatible with but not indicative of the diagnosis of TB, a positive IGRA test may suggest more strongly the possibility of a TB diagnosis.[54] However, the final decision should be based on clinical judgment.[54 60] IGRA tests cannot distinguish between active TB and LTBI.[60]

Advantages of IGRA tests

- It only requires one visit from the patient compared to two visits for a TST
- IGRA demonstrate improved specificity over the TST i.e. the proportion identified as disease free and the reduced cross-reactivity with BCG vaccine and most NTM means that persons are less

likely to have unnecessary treatment for presumed LTBI if they are correctly identified as disease free

- IGRA are at least as sensitive as the TST.[75]

Limitations of IGRA tests

- Some patients may find a blood test less acceptable than an intradermal test
- The TST is cheaper than IGRA
- Logistical issues may arise, as the laboratory must receive the blood sample within 8 hours for the T-SPOT.TB test and within 16 hours for QuantiFERON-TB Gold® In-Tube assay. [75]

Recommendations on IGRA use are based on the best currently available scientific evidence and medical practice. Over time, if new evidence emerges in relation to IGRA, this will be reviewed by the committee and the recommendations revised if deemed appropriate.

2.7 Interpretation of TST and IGRA Results

Use of the following table (from the Public Health Agency of Canada)[60] is recommended for the interpretation of TST and IGRA results:

Table 2.2: Interpretation of results when both TST and IGRA results are available

Risk of developing TB disease if infected with *M. tuberculosis***						
	High			Low		
	IGRA positive	**IGRA negative**	**IGRA indeterminate**	**IGRA positive**	**IGRA negative**	**IGRA indeterminate**
TST +ve	Consider treatment for LTBI			Consider treatment for LTBI	Treatment for LTBI is not necessary	Repeat IGRA test or base interpretation on TST result
TST -ve	Consider treatment for LTBI	LTBI treatment not necessary if immuno-competent	Repeat IGRA test or base interpretation on TST result	Consult TB specialist	Treatment for LTBI not necessary	

Source: Updated recommendations on interferon gamma release assays for latent TB infection. Canada Communicable Disease Report, Public Health Agency of Canada (October 2008). Reproduced with the permission of the Minister of Public Works and Government Services Canada, 2009. Available at www.phac-aspc.gc.ca/publicat/ccdr-rmtc/08pdf/acs-6.pdf.

** See table 3.3 in chapter 3 for risk factors for the development of active TB disease among persons with LTBI

Note:

- This table is offered in the context of the IGRA recommendations above and is not meant to be a comprehensive guide to the management of LTBI. See chapter 3 for the management of LTBI and for groups at risk of developing active TB disease (table 3.3)
- IGRA are more specific in populations that include previously BCG vaccinated individuals and hence would be very useful in the Irish context as it would lead to a decrease in false positive results.

2.8 Chest X-Ray

Chest X-Ray for the diagnosis of LTBI

Chest radiography is not usually considered a tool to diagnose LTBI. Its main role is in the diagnosis of TB disease. However, it is quite common that a chest X-ray is done for some other reason and radiographic abnormalities consistent with previous TB infection are detected. Individuals are considered to have inactive TB or LTBI when their chest X-ray shows certain abnormalities consistent with TB infection AND they have a positive TST result (table 2.1). These individuals have an increased risk for reactivation and may be considered for treatment of LTBI (chapter 3).

The following radiographic findings are commonly believed to represent latent/inactive TB. While some are associated with increased risk of reactivation of active TB disease in future, others are not.

- **Granulomas that may be calcified or not:** this doubles the risk of reactivation resulting in active TB disease
- **Calcified hilar lymph nodes:** if there are no parenchymal lesions, these individuals do not appear to have an increased risk relative to those who are TST positive and have normal chest X-rays
- **Costophrenic angle blunting:** this is due to past pleural effusion or pleurisy which may have many causes. The most common cause in individuals from countries with high TB incidence and other TB-endemic areas is previous exposure to *Mycobacteria tuberculosis.* Such individuals have an increased risk of reactivation
- **Apical pleural capping:** this is not considered to be related to TB infection and is a non-specific finding that is more common in older individuals
- **Apical fibronodular disease:** this is associated with increased risk of reactivation ranging from 6 to 19 times greater than those who are TST positive and have normal chest X-rays. Individuals with more extensive abnormalities have greater risk of disease.[30]

Source: Canadian Tuberculosis Standards, 6th Edition. Public Health Agency of Canada, 2007. Reproduced with the permission of the Minister of Public Works and Government Services, 2009. Available at www.phac-aspc.gc.ca/tbpc-latb/pubs/pdf/tbstand07_e.pdf

Chest X-ray for the diagnosis of TB disease

Chest radiography [posterior-anterior (PA)][76] is the usual first step in the evaluation of an individual with pulmonary symptoms. Additional views may be ordered at the clinician's discretion. It is also recommended that patients suspected of having extrapulmonary TB have a chest X-ray to rule out pulmonary disease. In all instances, if the chest X-ray results are abnormal (including pleural TB), sputum samples should be collected on 2 to 3 separate days and tested for acid fast bacilli (AFB) (smears) as well as for culture and drug susceptibilities.

The radiological findings usually seen on chest X-ray for both immunocompetent and immunosuppressed adults are outlined below. However, it is important to be aware that chest X-ray has substantial limitations in the diagnosis of pulmonary TB disease.

Typical findings: a triad of classic findings are seen in immunocompetent adults

Position- apical-posterior segments of upper lobes or superior segment of lower lobes in 90%

Volume loss- this is a hallmark of TB disease as a result of its destructive and fibrotic nature

Cavitation- this is seen at a later stage and depends upon a vigorous immune response. Therefore, it may not be seen in severely immunocompromised individuals.

Atypical features:

These will be seen in patients with **immunocompromising conditions** such as HIV infection, diabetes, renal failure or corticosteroid use.

Hilar and mediastinal lymphadenopathy, particularly in HIV-infected individuals

Non-cavitary infiltrates and lower lobe involvement

Radiographic signs of complications:

Endobronchial spread of disease. TB may spread via airways to the ipsilateral and contralateral lower lobes. This results in irregular poorly defined small nodular shadows which represent acinar shadows. These will slowly enlarge and coalesce to form TB pneumonia, formerly known as "galloping consumption"

Pleural effusion can be seen concomitant with pulmonary disease and may represent TB empyema

Pneumothorax can rarely occur as a result of erosion of a caseous focus into a bronchus and simultaneously into the pleural space causing a bronchopleural fistula.

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Limitations of chest radiography

Sensitivity: chest radiography will have a sensitivity of only 70% to 80% for diagnosis of active TB based on the abnormalities listed above. If *any* abnormality is considered it will have more than 95% sensitivity. Approximately 10% of HIV-positive persons or close contacts with active pulmonary disease will have normal X-rays

Specificity is relatively poor, in the range of 60% to 70%. If the sensitivity were improved (any abnormality considered possible TB), then the specificity would be much lower

Inter reader variability: one of the greatest problems with chest X-ray reading is that the interpretation is highly variable. There is very poor agreement between readers regarding the presence of cavitation, hilar lymphadenopathy and the likelihood of active disease.[30]

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Recommendation:

Chest X-ray is not considered the gold standard for the diagnosis of pulmonary TB.

Chest X-ray in pregnancy

The decision to perform a chest X-ray on women undergoing evaluation for active TB disease during pregnancy should be made on a case-by-case basis following discussion between the respiratory physician/ infectious disease consultant and the consultant radiologist. A lead shield should be used if a chest X-ray is performed.[77]

Chest X-ray in children

Chest X-ray is useful in the diagnosis of TB in children. Children should have an anterior-posterior chest X-ray which should be read by a radiologist experienced in paediatric radiology. A lateral chest X-ray is taken only in certain cases and generally after consultation with a radiologist. In the majority of cases, children with pulmonary TB have chest X-ray changes suggestive of TB. The most common finding is persistent opacification in the lung in conjunction with enlarged hilar or subcarinal lymph glands. A miliary pattern of opacification in HIV-uninfected children is highly suggestive of TB. Patients with persistent

opacification which does not improve after a course of antibiotics should be investigated for TB. More than half of children with radiological pulmonary disease are asymptomatic (identified through contact tracing). The chest X-ray is typically "sicker" than the child.[77]