

Testing for tuberculosis

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Summary

Tuberculosis is caused by *Mycobacterium tuberculosis*. The approach to testing for tuberculosis depends on whether the aim is to diagnose active disease or latent infection. If active disease is suspected, it is important to identify the site of disease. Analysis of sputum specimens for mycobacteria should precede other tests. An infection should never be diagnosed as latent until active disease has been excluded. Tuberculin skin testing is recommended for diagnosing latent infection, but interferon gamma release assays may be useful in some circumstances.

Key words: diagnostic imaging, interferon gamma release assays, tuberculin skin tests.

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Introduction

Approximately 1000 new cases of tuberculosis (or TB) are diagnosed in Australia each year. Most of these patients were infected overseas and recent transmission within Australia is rare and limited to small clusters. Nevertheless, primary care clinicians need to remain aware of tuberculosis because early diagnosis and treatment prevents transmission.

Screening for latent tuberculosis is recommended before prescribing immunosuppressive therapy such as tumour necrosis factor alpha inhibitors, cancer treatment and transplantation. Patients with a high risk of tuberculosis reactivation (see Table 1), particularly those with HIV infection, should also be tested for tuberculosis.

Natural history of tuberculosis (Fig. 1)

Tuberculosis in humans is mainly caused by *Mycobacterium tuberculosis*. The infection is transmitted by respirable droplets generated during forceful expiratory manoeuvres such as coughing. Tuberculosis infection can be either active or latent. People with **active infection** have signs or symptoms caused by actively replicating tubercle bacilli. If this is in

the lungs they are potentially contagious and usually have symptoms such as cough, chest pain, shortness of breath, fatigue, weight loss, fever and night sweats. Those with **latent infection** have previously been infected but have no symptoms or evidence of disease and are not contagious. However, they remain at risk of developing active tuberculosis (reactivation) during their lifetime.

Various factors are associated with an increased risk of becoming infected and subsequently developing disease (Table 1 and Fig. 1). Transmission is most efficient in poorly ventilated, crowded environments. Droplets become diluted once they enter the external environment and *M. tuberculosis* is rapidly destroyed by ultraviolet radiation.

Following lung infection, multiplication and dissemination of the organism is contained once cell-mediated immunity develops at 2–12 weeks. The risk of an individual progressing to active disease in the months and first few years after infection depends on the bacterial load and the effectiveness of their immune defences. A depressed immune response at the time of infection increases the risk for progressive primary (including disseminated) disease.

If someone is already infected, the risk for reactivation increases when their immunity is low. In the absence of reinfections, disease occurring more than 5–7 years after infection usually follows a decline in cell-mediated immunity, including age-related waning of cell-mediated immunity and iatrogenic immunosuppression (Table 1).

Diagnostic tests for tuberculosis

Various investigations can be used to help diagnose tuberculosis. These include medical imaging, microbiology tests, tests of a patient's immune response (tuberculin skin testing and interferon gamma release assays) and histopathology.

Chest radiology

If a patient has no respiratory symptoms, a normal chest X-ray almost excludes pulmonary tuberculosis. Chest X-rays are valuable for detecting pulmonary lesions of tuberculosis, however activity of disease cannot be judged with certainty.

Table 1

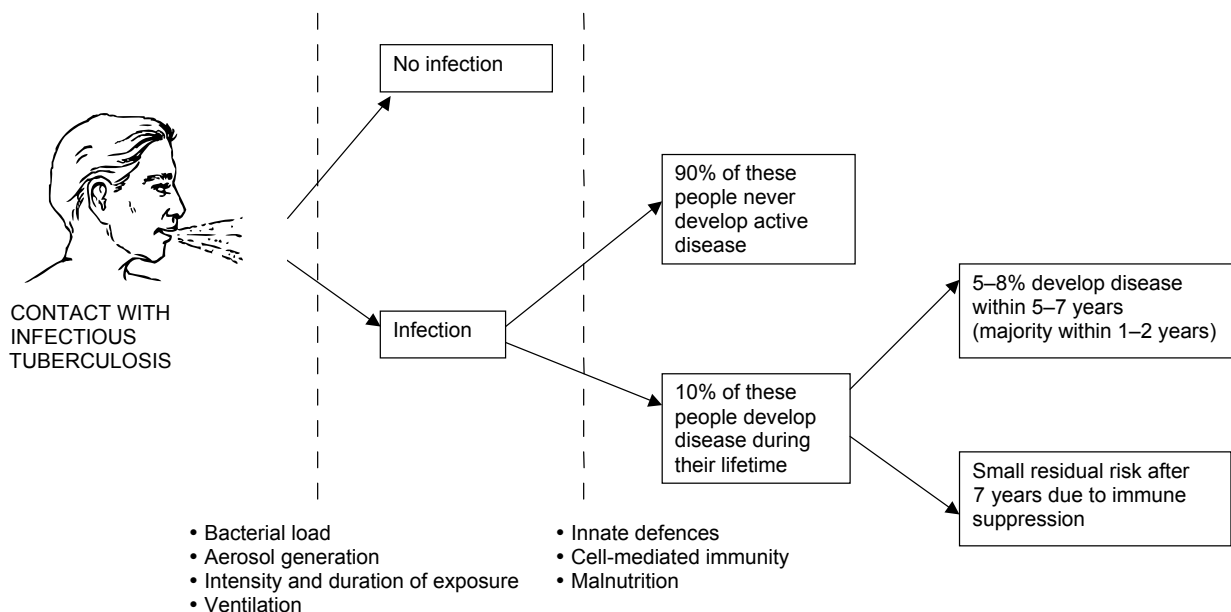
Risk factors for tuberculosis in Australia

Increased risk* of tuberculosis infection (i.e. increased risk of exposure to infectious tuberculosis)	<p>Migrants from high tuberculosis prevalence countries</p> <p>Members of Aboriginal and Torres Strait Islander communities with high incidence of tuberculosis</p> <p>Healthcare workers</p> <p>Household contacts (particularly children) of people at increased risk for tuberculosis</p>
Increased risk† of tuberculosis developing after infection‡	<p>HIV infection</p> <p>Silicosis</p> <p>Diabetes mellitus</p> <p>Chronic renal failure/haemodialysis</p> <p>Gastrectomy/jejunoileal bypass surgery</p> <p>Organ transplantation requiring immunosuppression</p> <p>Carcinoma (particularly head and neck carcinoma)</p> <p>Immunosuppressive therapies (corticosteroids, cytotoxic chemotherapy, tumour necrosis factor alpha inhibitors)</p> <p>Malnutrition and low body weight (≥10% less than ideal)</p> <p>Infancy</p> <p>Older age</p>

- * In other countries, residents of institutions (prisons, nursing homes), homeless people, users of illicit intravenous and other drugs (especially when associated with HIV infection), and impoverished populations with limited access to medical services have high incidence of tuberculosis infection. In general, the risk for these populations has not been as great in Australia with the exception of Aboriginal and Torres Strait Islander populations.
- † Most of this risk is related to cellular (T lymphocyte) immune defects.
- ‡ Patients with infections acquired within one year or with chest X-ray findings of fibrotic lung lesions consistent with untreated inactive tuberculosis have much greater risk of tuberculosis than those with tuberculosis infection acquired more than seven years previously.

Fig. 1

Natural history of tuberculosis in newly infected contacts



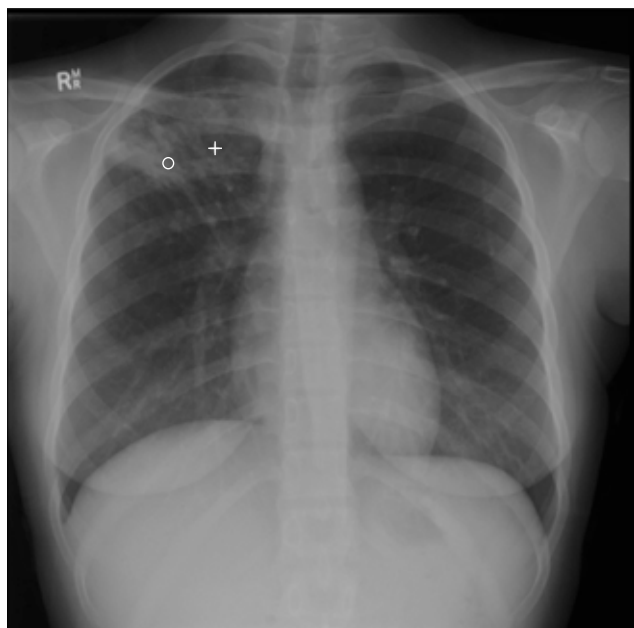
Classic upper zone chest X-ray changes (Fig. 2) can be due to other pathology, and pulmonary tuberculosis can have many other non-classic presentations with broad differential diagnoses. Unusual chest X-ray presentations (including normal chest X-ray) are more common in people with immune deficiencies and other comorbidities. Once pulmonary tuberculosis is suspected, the most appropriate initial investigation is sputum analysis and not further imaging, even if chest X-ray shows fibrosis which appears to be radiologically inactive.

Culture

Identifying *M. tuberculosis* remains the definitive means for diagnosis of active tuberculosis. Although culture of *M. tuberculosis* from a specimen is a sensitive test (75–80%), bacteria can take up to six weeks or more to grow. Collection of specimens should include three morning sputa whatever the suspected site of disease, unless chest X-ray is normal and there are no respiratory symptoms in a person with localised extrapulmonary disease.

Fig. 2

Chest X-ray showing pulmonary tuberculosis



Chest X-ray of an 18-year-old female who was part of a cluster of cases involving indigenous people in south-east Queensland and northern New South Wales. She presented with a history of cough for six months followed by weight loss, fevers, night sweats and fatigue. Sputum was smear-positive for acid-fast bacilli and grew *M. tuberculosis*. The X-ray shows an extensive infiltrate in the upper lobe of the right lung with air-space consolidation (note air bronchogram ○) and the formation of a number of cavities (+). There are surrounding reticulonodular satellite lesions and fibrosis of the involved lung with traction of the right upper hilum.

Smear microscopy and nucleic acid amplification

Mycobacteria retain certain dyes after being treated with acid and are classified as acid-fast bacilli. After collection, specimens can therefore be smeared on a slide, stained and visualised under the microscope. Although this technique, along with nucleic acid amplification, allows early identification it fails to detect many culture-positive cases. Nevertheless, microscopy for acid-fast bacilli rapidly identifies the most infectious tuberculosis cases and a positive sputum smear is sufficient for provisional diagnosis of tuberculosis.

When smears are positive for acid-fast bacilli, nucleic acid amplification of *M. tuberculosis* DNA can be used to rule out nontuberculous mycobacteriosis. This test has almost 100% specificity and sensitivity in acid-fast bacilli positive smears, with results provided within a few days (and potentially on the same day). While a negative nucleic acid amplification test of acid-fast bacilli almost excludes tuberculosis, the test can rarely be falsely negative in pulmonary tuberculosis (Fig. 3). Sputum smear-positive pulmonary tuberculosis is infectious so it is important to maintain infection control procedures while awaiting culture confirmation regardless of the nucleic acid amplification test result.

Screening for latent tuberculosis infection

The Australian National Tuberculosis Advisory Committee recommends tuberculin skin testing as the standard test for latent tuberculosis infection with targeted use of interferon gamma release assays (Quantiferon Gold) when high specificity is desired.

These tests have no role in initial investigations for active tuberculosis because negative results do not exclude disease and positive results may not necessarily indicate disease.

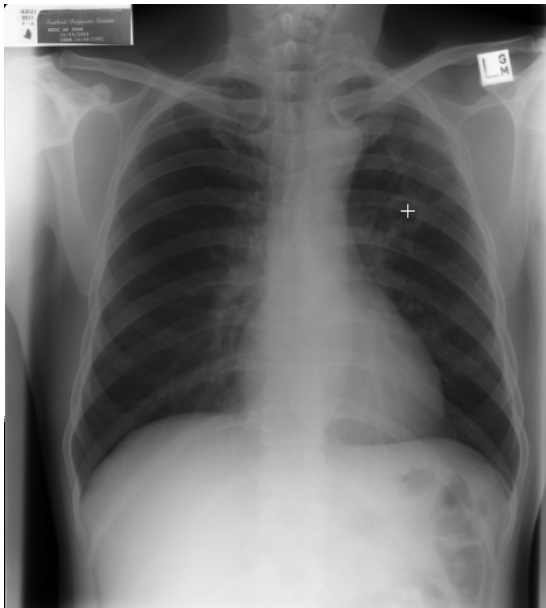
Tuberculin skin testing^{1,2}

This test measures a patient's immune response to *M. tuberculosis* antigens (tuberculin). A small amount of tuberculin is injected intradermally and the skin reaction is measured two or three days later (Fig. 4).

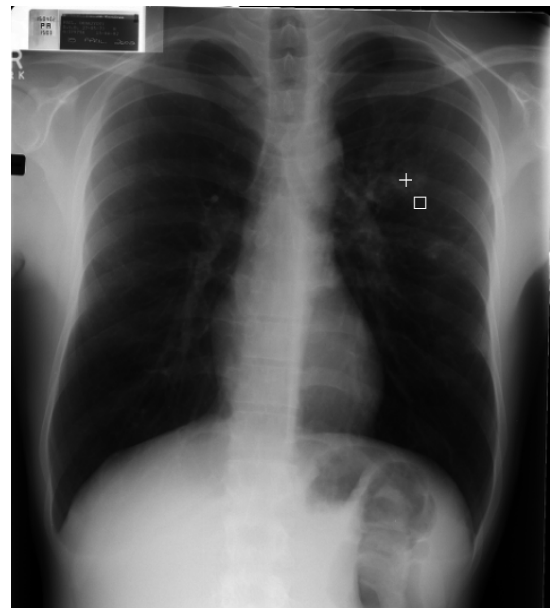
The test is very sensitive for detecting tuberculosis in healthy individuals if 5 mm induration is used to define a positive reaction. However, many conditions result in false negative reactions, including active tuberculosis (Box 1, part A). Bacillus Calmette-Guérin (BCG) vaccination and exposure to environmental nontuberculous mycobacteriosis cause intermediate size reactions (Box 1, part B). Sensitivity is often sacrificed by choosing larger indurations to define a positive reaction based on the incidence of tuberculosis and the extent of non-specific cross-reactivity in the population being tested. Box 2 provides general recommendations for categorising skin reactions, but regional tuberculosis control units should be consulted for local guidelines.

Fig. 3

Chest X-ray showing examples of sputum smear-positive tuberculosis with negative nucleic acid amplification test



A. Chest X-ray of 51-year-old male who arrived in Australia 15 years earlier from Vietnam. The X-ray was taken for investigation of unrelated shoulder pain and shows a cavity (+) adjacent to the left hilum. Sputum was smear-positive for acid-fast bacilli, however nucleic acid amplification was negative for *M. tuberculosis*. This was presumably because the organism lacked the IS6110 DNA insert which was the target of the test.



B. Routine chest X-ray taken for visa purposes in a 28-year-old university student from India. The X-ray showed a small cavity (+) with some surrounding infiltrate (□) adjacent to the left upper hilum. Initial sputum samples collected were smear-positive for acid-fast bacilli, but were repeatedly negative by nucleic acid amplification testing. Sputum samples were subsequently repeated. These specimens were more heavily smear-positive and tested positive for *M. tuberculosis* by nucleic acid amplification. The negative results were most likely due to a sampling error during collection of the first sputum specimen.

Fig. 4

Tuberculin skin testing, Mantoux method



A. Intradermal injection of tuberculin



B. Measuring induration 72 hrs later. Note: only the diameter of induration should be read, not the diameter of erythema.

Box 1

Factors that influence interpretation of tuberculin skin tests

A. Factors that may decrease skin reaction or give false negative reactions

Infections

Viral (e.g. HIV infection, measles, mumps, chickenpox)

Bacterial (e.g. pertussis, brucellosis, leprosy, overwhelming tuberculosis, pleural tuberculosis)

Fungal

Live virus vaccination (e.g. measles, mumps, polio)

Metabolic disease (e.g. chronic renal failure)

Malnutrition/protein depletion

Lymphoid neoplasms (e.g. Hodgkin's disease, lymphoma, chronic lymphocytic leukaemia)

Sarcoidosis

Drugs (corticosteroids, immunosuppressants)

Age (newborns and elderly)

Tuberculosis infection acquired within last eight weeks

Other conditions causing cell-mediated immune suppression

Local skin damage (dermatitis, trauma, surgery)

Incorrect handling and storage of tuberculin

Poor technique (related to intradermal injection or measuring induration)

B. Factors that may increase skin reaction or give false positive reactions

Exposure to or infection with nontuberculous mycobacteria

Past BCG vaccination

Trauma and irritation to site of intradermal injection before reading

Poor technique

BCG Bacillus Calmette-Guérin vaccination

As skin test reactivity can wane with time, two-step skin testing is sometimes used. If the initial skin test is not positive, it can be repeated within one or two weeks (to minimise the possibility of new tuberculosis infection influencing the re-test result) when antigen from the first test would have stimulated recruitment of memory T cells to the area. This will also boost non-specific reactivity from BCG and nontuberculous mycobacteriosis. It is used either to detect infections from the distant past, for example in older people being screened before starting immunosuppressive therapy, or to establish a baseline when repeat testing is planned to monitor for new tuberculosis infection.

Interferon gamma release assays

The non-specificity of tuberculin skin testing (Box 1) and the dependence on well-trained staff to minimise human error

Box 2

Criteria for defining a tuberculin skin testing reaction as positive *

≥5 mm – in people with recent exposure (within 2 years) to tuberculosis + high risk for progression to active disease (e.g. <5 years of age, HIV infection, other immunosuppressive illness; see Table 1)

≥10 mm – in people with recent exposure to tuberculosis, regardless of BCG vaccination status; all non-BCG vaccinated people except for those with both low lifetime risk for tuberculosis infection and residence in geographical areas where exposure to environmental nontuberculous mycobacteriosis is common

≥15 mm – in all people regardless of BCG vaccination status

* This refers to the induration produced by an intradermal injection of purified protein derivative (PPD) equivalent to 5 units of PPD-S. These criteria are meant as suggestions only. Local tuberculosis control units should be consulted for local guidelines.

BCG Bacillus Calmette-Guérin vaccination

are overcome by interferon gamma release assays. These laboratory tests are much more specific than tuberculin skin testing³⁻⁶ because the antigens used are expressed by *M. tuberculosis*, but not BCG or most nontuberculous mycobacteriosis (exceptions include *M. kansasii*, *M. marinum*, *M. szulgai* and *M. flavescens*). The current blood tests either measure the amount of interferon gamma released by lymphocytes or quantify the number of T lymphocytes releasing interferon, after incubation with *M. tuberculosis* antigens.

Interferon gamma release assays are at least as sensitive as tuberculin skin testing for detecting recently acquired latent tuberculosis infections and may be even more sensitive for detecting recently acquired active infections.⁵ Their increased specificity makes them useful in screening for recent tuberculosis infection in populations with a low incidence of tuberculosis and high uptake of the BCG vaccination. However, many studies show that tuberculin skin testing and interferon gamma release assays perform similarly in non-BCG vaccinated people at high risk for recent tuberculosis infection, if an appropriate cut-off (for example 10 mm induration) is used for tuberculin skin testing.⁵

It is not known if interferon gamma release assays are as sensitive as tuberculin skin testing for detecting remotely acquired (more than 5–10 years earlier) latent infections which may reactivate during immunosuppressive therapy. It is also suggested that interferon gamma release assays may be inferior to tuberculin skin testing in young children, particularly those under two years.³

Tuberculin skin testing does not require access to laboratory or phlebotomy so it is useful in remote settings and for infants and children. With well-trained staff, skin testing can be combined with counselling, education and clinical assessment for active tuberculosis. The distribution of tuberculin skin testing reactions in various populations^{1,2} is better understood than that of interferon gamma release assays. Performance of interferon gamma release assays has not been tested in geographical areas where subclinical infections due to nontuberculous mycobacteriosis such as *M. marinum* or *M. leprae* are common.

Histopathology

Pathological examination of biopsied tissue may support a diagnosis of tuberculosis when bacteriology is negative or cannot be done, however histology is non-specific. Always ensure enough tissue is available for culture if it is required.

The patient's risk of tuberculosis should be considered to avoid misclassifying non-caseating granulomatous processes due to tuberculosis as sarcoidosis, Crohn's disease, or other granulomatous disease. Similarly, caseating granulomas due to tuberculosis in cervical lymph nodes of young children may be misclassified as nontuberculous mycobacterial lymphadenitis.

Approach to diagnosis

The key to early diagnosis of tuberculosis is to consider the possibility that a patient may be infected.

Active tuberculosis

If active infection is suspected in an adult, sputum samples should be analysed for mycobacteria unless the chest X-ray is normal and there are no respiratory symptoms. Even if non-pulmonary tuberculosis is suspected, it is important to realise that patients may also have pulmonary tuberculosis which is responsible for transmission of tuberculosis. Other testing (including further medical imaging, immunological tests or bronchoscopy) can then be carried out in consultation with a specialist.

Children rarely present with infectious tuberculosis and often have smear- and culture-negative tuberculosis even with severe forms of tuberculosis such as meningitis or miliary disease. Thus, early referral to a paediatrician or tuberculosis service is required in a child at high risk who is failing to thrive or is lethargic and listless.

Latent infection

Screening for latent tuberculosis is best carried out by clinicians who can exclude active tuberculosis and manage latent tuberculosis. The choice of tuberculin skin testing or

an interferon gamma release assay will depend on local availability. Clinicians who are experienced in interpreting tuberculin tests and involved in population screening are likely to use tuberculin skin testing as the preferred test, using interferon gamma release assays when required for specificity.

Tuberculin skin test readings are interpreted after considering the clinical and epidemiological setting rather than defining a specific positive or negative cut-off. Skin testing by trained staff is done in conjunction with patient education, counselling, and screening for symptoms of tuberculosis.

Interferon gamma release assays will be preferred by clinicians assessing individual patients within a diverse practice. There is no need to refer the patient, as with the specialist tuberculin skin test. The result will be reported as positive, negative or indeterminate and not require integration of further epidemiological or clinical information.

As interferon gamma release assays are more specific, they are superior to tuberculin skin testing in people with a low lifetime risk for tuberculosis or with previous BCG vaccination. With trained staff, tuberculin skin testing lends itself to community screening and in populations at high risk for tuberculosis and it may be more sensitive for detecting remote (rather than recent) tuberculosis infections.

The best approach may integrate both tests and requires further study.* Whatever approach is used to diagnose infection, it is important to exclude active tuberculosis before considering the infection latent and offering preventive treatment.

Conclusion

It is clear that tuberculosis remains a major cause of disease globally, and many immigrants to Australia come from countries where tuberculosis is prevalent. It is therefore important for clinicians to maintain an appropriate index of suspicion about this disease.

Early diagnosis and effective management of active tuberculosis remain the most effective strategies for public health control of tuberculosis. As pulmonary tuberculosis is infectious, it is particularly important to consider the possibility of tuberculosis in patients with subacute and chronic infectious syndromes and with a cough for longer than two to three weeks. If such a patient has an abnormal chest X-ray, analysis of three morning sputum specimens will rapidly detect those with active transmissible infection. Tuberculin skin tests and interferon gamma release assays have no role in the initial investigation for active pulmonary tuberculosis. They are mainly used for detecting latent tuberculosis in people when active tuberculosis has been excluded, and for whom preventive treatment would be considered.

* See proposed approach (Fig. 5) online with this article at www.australianprescriber.com/magazine/33/1/12/18

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Further reading

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Conflict of interest: none declared

Self-test questions

The following statements are either true or false (answers on page 27)

1. Sputum culture is the definitive investigation for diagnosing latent tuberculosis.
2. A negative tuberculin skin test rules out the possibility of active tuberculosis.

Guidelines for thromboembolism prophylaxis in hospitals

New Australian guidelines for preventing venous thromboembolism are now available.¹ These guidelines give evidence-based recommendations for adult patients including pregnant women. Drugs covered by the guidelines include the heparins², fondaparinux, danaparoid, rivaroxaban, dabigatran etexilate, aspirin and warfarin. Mechanical options such as graduated compression stockings are also considered.

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