Tuberculosis diagnostics in active and latent disease

Every year approximately 10 million people fall ill and 1.7 million die from tuberculosis worldwide (World Health Organization, 2017a), from about 2 billion latent infections (Gideon and Flynn, 2011). In England in 2016 there were 5664 nationally notified cases, 74% of which involved individuals born outside the UK, the majority in large urban centres like London (39%) (Public Health England, 2017).

Tuberculosis is predominantly caused by *Mycobacterium tuberculosis* bacilli, although other pathogenic species also exist. An infectious individual generates bacilli-containing aerosols mainly by coughing, resulting in transmission by inhalation in about 20–30% of close contacts. The bacilli are ingested by and proliferate within resident lung alveoli macrophages of the new host, which may migrate from the lungs to other tissues. In 90–95% of cases this results in containment of the infection through cell-mediated immune responses and granuloma formation, termed latent tuberculosis infection.

Active disease occurs when the immune system is unable to contain the bacilli, simply referred to as 'tuberculosis'. Infection may progress to active disease at the site of infection as pulmonary tuberculosis, or other sites as extrapulmonary tuberculosis by haematogenous or lymphatic spread. The cell-mediated immune response that develops after infection is not sufficient to eradicate the bacilli and therefore latent tuberculosis infection always has a risk of future reactivation: about 5–10% within 2 years (early progression or primary tuberculosis) and 5% after 2 years (late progression) (Chaisson and Nachega, 2010). In truth, latent and active tuberculosis exist on a spectrum, with risk factors for progression discussed in the following section.

Several diagnostic tools are available, some of which are intended for the diagnosis of latent tuberculosis, others for active tuberculosis. This article dispels some misconceptions about tuberculosis diagnostics, outlines the different approaches required for active and latent infection, and highlights several grey areas. It focuses on tuberculin skin tests, interferon-gamma release assays, microbiological techniques, polymerase chain reaction and whole genome sequencing.

Diagnostic approach to active tuberculosis

The diagnosis of active tuberculosis relies on a combination of history, examination and the appropriate choice of investigations. Constitutional symptoms such as fever, drenching night sweats and weight loss in the right epidemiological context (*Table 1*) may suggest the diagnosis of active tuberculosis, but lymphoma must be kept in mind as the most common differential diagnosis. The presence of additional symptoms, generally lasting more than 2 weeks, will help guide further examination and investigations.

Risk factors for acquiring the infection, as well as risk factors for developing active disease, need to be assessed (*Table 1*). Healthy individuals with latent tuberculosis infection have a 0.1% annual risk of developing active tuberculosis, equating to a 10% lifetime risk, with the majority of active disease occurring within the first 2 years of infection (Schwartzman, 2002). Immunocompromised individuals, in comparison, have a 10% annual risk of developing active tuberculosis (Gideon and Flynn, 2011).

Active tuberculosis can affect many organ systems. The most straightforward diagnostic scenario is a chronic productive cough, apical opacities on the chest radiograph and *M. tuberculosis* isolated from sputum. Often, however, the diagnosis is challenging, as is the case in tuberculous meningitis, spinal tuberculosis, miliary tuberculosis, abdominal tuberculosis and genitourinary tuberculosis. A definitive diagnosis requires isolation of the causative organism from a wide range of potential clinical specimens (*Table 2*). The initial assessment will indicate which of these sites or tissues to target.

Microbiological methods

The definitive diagnosis of active tuberculosis is by identification of *M. tuberculosis* using

Table 1. Risk factors for tuberculosis transmission and development of active disease

Risk factors for acquiring tuberculosis infection (epidemiological factors)	Previous residence in tuberculosis-endemic countries
	Household or close contacts of patients with open tuberculosis (pulmonary and laryngeal tuberculosis)
	Health-care workers who have worked with patients with open tuberculosis
Risk factors for developing active tuberculosis	Immunocompromising conditions: HIV, lymphoma, cancer
	Immune-modifying medications: cancer therapies, tumour necrosis factor-alpha inhibitors, long-term steroid use
	Other associations: smoking, chronic kidney disease and diabetes mellitus

From Jasmer et al (2002), Gideon and Flynn (2011)

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Table 2. Clinical specimens from which *Mycobacterium tuberculosis* may be isolated

Spontaneous or induced sputum, or bronchoscopy washings
Pleural fluid or pleural biopsy
Abscess fluid
Lymph node aspirates or biopsy
Pericardial fluid or pericardial biopsy
Blood cultures
Bone marrow aspirates
CSF, brain biopsy
Bowel, liver or omental biopsy
Early morning urine samples
Skin biopsy

From National Institute for Health and Care Excellence (2016)

microscopy and culture of relevant samples of fluid or tissues, from suspected disease sites indicated from the history, examination and radiological findings. However, in some cases presumptive treatment is required, as a microbiological diagnosis is not always easy to obtain, especially in the case of cerebral tuberculosis and tuberculosis lymphadenitis.

Samples, such as sputum in the case of pulmonary disease, are examined using either an auramine stain with fluorescence microscopy or a Ziehl–Neelsen stain to detect acid-fast bacilli. Biopsy specimens may show caseating granulomas on histology, suggesting a diagnosis of tuberculosis, although bacilli are frequently absent on such preparations. Samples are also incubated in solid Löwenstein–Jensen (*Figure 1*) or liquid media (*Figure 2*) for up to 6 weeks to detect growth of mycobacteria. When grown in culture, mycobacteria are sent to a reference laboratory for speciation. Phenotypic drug sensitivity testing to first-line drugs is also performed.

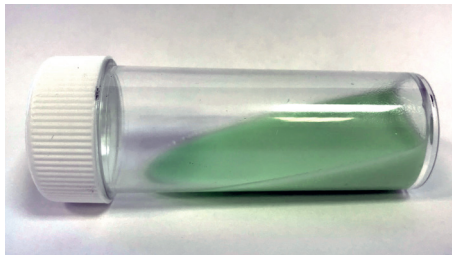

Figure 1. Löwenstein–Jensen solid culture medium for mycobacteria.

> “Biopsy specimens may show caseating granulomas on histology, suggesting a diagnosis of tuberculosis, although bacilli are frequently absent on such preparations.”

Molecular methods

Owing to the slow replication time of mycobacteria (Hett and Rubin, 2008) and the scanty number of mycobacteria present in some samples, a positive result may not be apparent for several weeks. Cepheid's GeneXpert offers a particular advantage by using automated polymerase chain reaction technology (*Figure 3*). Several centres, in London and elsewhere, also have their own 'in house' test for detecting *M. tuberculosis* DNA by polymerase chain reaction.

With a turnaround time of about 2 hours, using unprocessed sputum or other samples, the GeneXpert MTB/RIF is able to detect genetic material from *M. tuberculosis*. The latest generation polymerase chain reaction test, the GeneXpert MTB/RIF Ultra, has a sensitivity very close to that of liquid culture as shown in *Table 3*. It is thus very useful in immunocompromised patients, where bacterial numbers are often scanty. However, polymerase chain reaction tests simply detect genetic material and thus false positives may result from dead bacilli from previously cured tuberculosis, up to 7 years later (Stevenson et al, 2015).

The GeneXpert also detects presence of the rpoB gene mutation, which is associated with rifampicin resistance, currently a key drug in the first-line tuberculosis drug regimen. Isoniazid resistance accompanies rifampicin resistance in the majority of cases (Ormerod, 2005), making multidrug-resistant tuberculosis (resistance to both rifampicin and isoniazid) a possibility. If

Figure 2. Liquid culture medium for mycobacteria.

Figure 3. The GeneXpert cartridge for tuberculosis polymerase chain reaction testing.

Table 3. Comparison of the number of mycobacterial bacilli required to be present in a millilitre of sample for a positive result

Diagnostic method	Limit of detection per ml of sample
Liquid culture	10 bacilli
Solid culture	100 bacilli
Smear microscopy	5000–10 000 bacilli
GeneXpert MTB/RIF (first generation)	114 bacilli
GeneXpert MTB/RIF Ultra	16 bacilli

From Cuevas (2014), World Health Organization (2017b)

> “Advancements in processing techniques, reduced turnaround time and cost have enabled whole genome sequencing to develop from being an epidemiological research tool to a clinically usable diagnostic tool.”

M. tuberculosis is later grown in culture, phenotypic drug sensitivity testing can confirm drug resistance with the suppression of *M. tuberculosis* growth in culture in the presence of corresponding drugs.

Finally, whole genome sequencing is increasingly being used for the diagnosis of tuberculosis. Advancements in processing techniques, reduced turnaround time and cost have enabled whole genome sequencing to develop from being an epidemiological research tool to a clinically usable diagnostic tool, in particular through the identification of genotypic markers for drug resistance (Lee and Pai, 2017). The UK was the first country to make whole genome sequencing available as part of routine care in the spring of 2018.

Diagnostic approach to latent tuberculosis infection

The tuberculin skin test

The tuberculin skin test and interferon-gamma release assay are tests designed to detect latent tuberculosis infection (Pai et al, 2014). They are unable to identify active disease or predict the progression from latent infection to active disease (Ruhwald and Andersen, 2016). The tuberculin skin test, of which Mantoux is the approved administration method, is given as an intradermal injection of purified protein derivative (a distilled complex of *M. tuberculosis* antigens – *Figure 4*) into the forearm. The presence and size of induration is measured 48–72 hours later. Care must be taken not to measure the zone of erythema, which may be larger than the area of induration (*Figure 5*). The induration is the result of cell-mediated immune recognition from previous infection and response to *M. tuberculosis* antigens.

Predefined thresholds determine a positive test. The most recent National Institute for Health and Care Excellence (2016) guidelines have simplified recommendations, removing varying cut-off values for different patient groups and now define a positive test as ≥5 mm for all patients. However, local practice may vary as the lower cut-off reduces the specificity of the test. False positive and false negative results may occur for a number of reasons (*Table 4*). With regards to the bacillus Calmette–Guérin vaccine (BCG) vaccine, a false positive result is unlikely to occur after 5–10 years since the tuberculin skin test response to BCG antigens is unlikely to last beyond this timeframe (Gideon and Flynn, 2011).

The tuberculin skin test has been in use for over 100 years in one form or another and, as such, is inexpensive and has no need for laboratory infrastructure. The test result is also quantifiable, one review suggesting that larger areas of induration may be more predictive of active disease (Watkins et al, 2000). However, the large variation in risk associated with different induration sizes makes actual risk hard to quantify. Disadvantages include the need for a repeat patient visit for result reading, an experienced nurse or doctor to read the result, and cross-reactivity with the BCG vaccine and environmental non-tuberculous mycobacteria. The false positive result caused by environmental mycobacteria is more likely in areas of low tuberculosis prevalence (Pai et al. 2014), which includes most of the UK.

TOP TIPS

- In a patient with suspected pulmonary tuberculosis, hand-deliver respiratory samples to the laboratory for a same-day diagnosis as these are often delayed or sometimes lost in transit.
- Urgent analysis of specimens with polymerase chain reaction technology such as the GeneXpert (where available) will speed up the diagnosis of tuberculosis and give and early warning for drug resistance. The GeneXpert is only validated for respiratory samples but may be used on other samples and always requires authorization by the local infectious disease or microbiology team.
- When investigating for tuberculous meningitis, the diagnostic yield of a lumbar puncture is low, but can be significantly increased by sending 6 ml of CSF for tuberculosis polymerase chain reaction, microscopy and culture.

Figure 4. Purified protein derivative and insulin needle used in the Mantoux test.

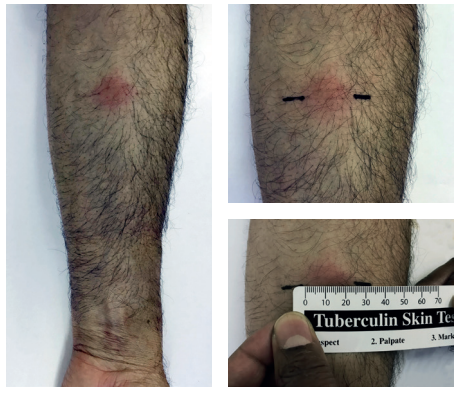

Figure 5. Reading of a positive Mantoux test: inspect, palpate, mark and measure the induration size.

Table 4. Pitfalls of the tuberculin skin test

False positive result	Previous exposure to environmental Mycobacteria
	Previous bacillus Calmette–Guérin (BCG) vaccine
False negative result	Immune suppression: HIV, extremes of age, immunosuppressive drugs (including steroids, anti-tumour necrosis factor, methotrexate)
	Anergy as a result of current active tuberculosis

Figure 6. Collection bottles for the QuantiFERON test.

Interferon-gamma release assays

In comparison to the tuberculin skin test, the interferon-gamma release assay test is more specific as it does not cross-react with antigens present in the BCG vaccine or environmental mycobacteria. While both the tuberculin skin test and interferon-gamma release assay have over 95% specificity in low tuberculosis incidence settings, the specificity of the tuberculin skin test drops as low as 60%, depending on timing and frequency of BCG vaccination (Pai et al. 2014). The interferon-gamma release assay is more expensive and requires a laboratory for processing.

The principle of the test is in the name: an assay of the amount of interferon-gamma released in response to stimulation by antigens found in the *M. tuberculosis* complex. There are two commercially available interferon-gamma release assays: the QuantiFERON and T-SPOT.TB test.

The QuantiFERON (Qiagen) is performed by drawing blood into four tubes (*Figure 6*) containing one of two different antigens specific to *M. tuberculosis* (ESAT-6 and CFP-10), a positive control (the mitogen), and a negative control for subtraction of background interferon-gamma activity. The amount of interferon-gamma released by T-cells is measured, serving as an indicator of the cell-mediated immune response to tuberculosis and thus a proxy indicator of infection, whether latent or active.

Another laboratory-based test of interferon release is the T-SPOT.TB (Oxford Immunotec) test, in which mononuclear cells are separated, counted and then incubated with the same antigens used in the QuantiFERON. The number of interferon-gamma producing T-cells are then counted using an enzyme-linked immunosorbent spot assay. A predetermined threshold determines a positive result. The T-SPOT.TB test appears to have a higher sensitivity than both the tuberculin skin test and the QuantiFERON (90% for the former and 80% for the latter two tests) (Pai et al, 2014). The choice of test for latent tuberculosis infection depends largely on local availability.

Conclusions

The diagnosis of tuberculosis relies on a good history, and an understanding of epidemiological risk, thorough examination and carefully chosen investigations. Active tuberculosis is diagnosed by compatible clinical and radiological features, as well as microbiological confirmation in the form of microscopy, culture and molecular methods. Tests such as the tuberculin skin test and interferon-gamma release assay are supplementary in the diagnosis of active tuberculosis, and may be useful for their negative predictive value in difficult cases when molecular tests and culture remain negative, in which case specialist input is advised because of the potential diagnostic pitfalls. **BJHM**

KEY POINTS

- It is helpful to think about tuberculosis in terms of latent and active disease.
- The gold standard of active tuberculosis diagnosis is with positive microscopy and culture of relevant tissue samples, but it may also be diagnosed on molecular detection or treated empirically.
- History, examination and focused radiology will most often indicate which samples to obtain for a microbiologically confirmed diagnosis of tuberculosis.
- Tuberculin skin test and interferon-gamma release assay are mainly for the identification of latent disease, but it may be used in some cases of suspected active disease by specialists in the field and interpreted cautiously.
- Tuberculosis polymerase chain reaction testing is useful as an early indicator of drug-resistant tuberculosis.
- Identify risk factors for tuberculosis transmission and risk factors for development of active disease, in particular immunocompromise.

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