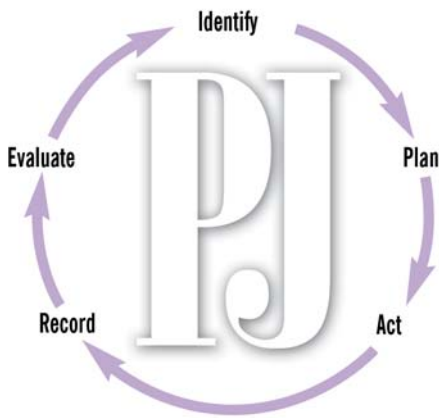


How tuberculosis can be diagnosed

In this second article on tuberculosis, **Helen Booth** describes the diagnosis of active or latent disease as part of a tuberculosis control programme



Identify knowledge gaps

1. How is active tuberculosis usually diagnosed?
2. How is latent tuberculosis usually diagnosed?
3. Which drugs predispose patients to a higher risk of tuberculosis?

Before reading on, think about how this article may help you to do your job better. The Royal Pharmaceutical Society's areas of competence for pharmacists are listed in "Plan and record", (available at: www.rpsgb.org/education). This article relates to "common disease states and their drug therapies" (see appendix 4 of "Plan and record").

A core element of any effective tuberculosis (TB) control programme is to diagnose cases with active disease promptly and initiate effective therapy. The aim is to reduce individual morbidity and mortality that can result from delays in diagnosis and also to break the cycle of transmission. Early identification of pulmonary TB is critical to prevent other people from being infected. Mortality from TB in the UK is between 5 and 10 per cent.

An increasingly emphasised strategy for TB control is the identification of people with latent TB. People who have been infected with TB and have initially contained the infection run a risk of developing active disease in the future and would benefit from treatment.

Diagnosis of active TB

Active TB should be considered in most differential diagnoses. This is because symptoms are often non-specific (eg, weight loss and night sweats) and depend on which part of the body is affected. The possibility of TB must always be considered in people at higher risk of the disease. They include:

- Those who have been in contact with TB cases (including health care professionals)

- Those with reduced immunity (particularly people infected with human immunodeficiency virus, and also patients being treated with immunosuppressants or with renal failure or diabetes)
- Those from countries with a high prevalence of TB
- Those with socioeconomic risk factors (eg, homelessness, alcoholism, intravenous drug use)

The longest delays in diagnosis often occur in cases from low prevalence areas and without obvious risk factors because the possibility of TB is often forgotten. All health care professionals, therefore, have a responsibility to be aware of TB. Health care workers also have a duty to be checked promptly if they have clinical symptoms suggestive of active TB, especially if they work with immunocompromised patients.

Chest x-ray Because TB is commonly pulmonary (80 per cent of cases) and symptoms can be non-specific, it is often the chest x-ray that first suggests TB. The disease typically presents as shadowing, with or without cavitation, in the upper lobes. However, other abnormalities, such as fluid around the lungs (pleural effusion), enlargement of the mediastinal lymph nodes or miliary shadowing (classically described as shadows like small millet seeds throughout the lungs), can sometimes be seen. In HIV-positive patients chest x-rays may not be characteristic of TB.

Microbiological investigation Growing or culturing *Mycobacterium tuberculosis* (MTB) from samples is the gold standard for diagnosing TB. Clinical samples relevant to the site under investigation (eg, multiple sputum samples in suspected pulmonary TB and lymph node aspirate or biopsy in suspected lymph node TB) are sent to hospital microbiology departments. The samples are then put through four main steps unique to the investigation of TB (see Figure 1, p317).

Smear The first step is to perform a smear test. The sample is stained (eg, using auramine-phenol) and the organism looked for under a microscope. The importance of the smear test is that not only will it provide results within 24 hours but it describes a subset of patients ("sputum smear-positive cases") considered to be the most infectious.

It is estimated that more than 5,000 organisms per ml of sputum are needed to be visible under a microscope. Patients who are

Summary

- The symptoms of active TB are often non-specific
- Increasing rates of TB in specific groups — some areas of London have rates of TB similar to those in Delhi — raises the importance of active case finding
- Culture of *Mycobacterium tuberculosis* from clinical samples remains the gold standard for diagnosis
- Increasing rates of drug resistance highlight the need for clinical samples to be obtained for sensitivity testing
- Newer molecular biological techniques for identifying MTB DNA, specific immune responses to MTB and for mutations in MTB DNA are exciting developments that could provide vital information and improve the clinical management of TB

sputum smear-positive, therefore, have a higher bacterial load in their sputum and are likely to be more infectious than patients with pulmonary TB who are sputum smear-negative.

Panel 1: Nucleic amplification techniques

The basis of nucleic amplification (NA) techniques is to multiply a segment of DNA present in a sample to a level at which its presence can be identified.

Identifying active tuberculosis If the DNA segment is unique to *Mycobacterium tuberculosis* (MTB) it can confirm the presence of MTB and differentiate it from mycobacteria other than tuberculosis (MOTTs). These tests are clinically most useful in previously untreated people who are sputum smear-positive but in whom MOTTs is a significant possibility (eg, in patients who are HIV positive or have chronic lung disease).

Sensitivity of sputum smears is about 50 per cent compared with 80 per cent and 100 per cent for NA assays and culture, respectively. NA assays are expensive. They could be used if there is a high suspicion of TB but the smear is negative and a positive result would change management, or if a failure to diagnose TB promptly could have devastating clinical consequences (eg, in TB meningitis).

Rapid detection of drug resistance Resistance to rifampicin is usually associated with mutations in the region of the *rpoB* gene and these can be detected with NA techniques.

Helen Booth, MBBS, FRCP is a consultant thoracic physician at University College London Hospital

Culturing *Mycobacterium tuberculosis* is the gold standard for diagnosing the disease

Culture and identification Smears do not differentiate MTB from other mycobacteria (eg, *M kansasii* or *M avium* complex), so the next investigative steps are to culture and identify the *Mycobacterium* species. Löwenstein-Jensen media, first used to culture mycobacteria in the 1930s, is still widely used but growth can take up to eight weeks. Fully automated liquid culture systems that detect mycobacterial growth within two or three weeks, by means of a fluorescent marker, have been developed, but there is a need for even quicker tests. New molecular biological techniques, such as nucleic amplification (see Panel 1, p316) and ELISPOT (see Panel 2, p318), are emerging and these can be used to differentiate between MTB and mycobacteria other than tuberculosis (MOTTs) more rapidly.

Compared with sputum smears, only 200 organisms per ml are needed for a positive culture. Sputum smears are positive in between 50 and 80 per cent of culture confirmed cases of pulmonary TB.

Drug sensitivity testing The final stages of identification of the *Mycobacterium* species and drug sensitivity testing are performed on positive cultures at regional reference laboratories rather than in hospital laboratories. Early identification of MTB resistant to rifampicin is important because rifampicin is one of the main first line drugs for the treatment of TB. In addition, rifampicin resistance can also be a marker for the presence of multi-drug resistant TB (TB resistant to both rifampicin and isoniazid) and super-resistant TB (TB resistant to rifampicin, isoniazid and at least one second line drug). Increasing rates of drug resistance mean that it is important to perform TB culture and sensitivity testing wherever possible.

Diagnosis in practice Microbiological identification of TB (the gold standard), only occurs in about 60 per cent of all notified cases. Reasons for the absence of microbiological confirmation include:

- TB was not considered in the diagnosis so no samples were sent but histological examination shows granulomatous inflammation, of which TB is a differential diagnosis (2 per cent of notified cases are thought to have laboratory support for the diagnosis based on histological findings alone¹)
- There is a strong clinical indication of TB but it is considered too invasive to obtain samples, for example, from a child who has no cough but is in contact with a sputum smear-positive mother and has a chest x-ray suggestive of TB
- Samples were culture negative (this may reflect “pauci-bacillary disease” [only a small number of MTB organisms are present], sampling error or technical problems)

The diagnosis of active TB in a significant proportion of patients, therefore, is still based on clinical features, radiology and response to treatment. Tuberculin skin tests (TSTs, see

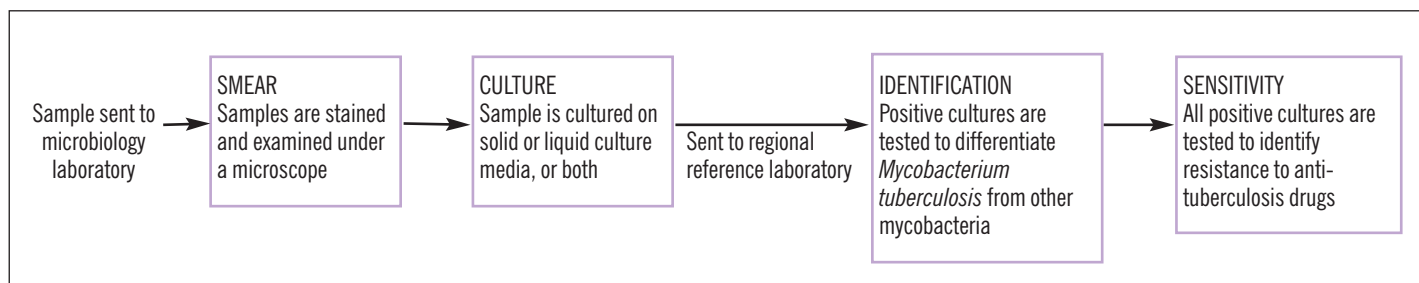


Figure 1: Four stages of microbiological diagnosis of tuberculosis

later) have a limited role in the diagnosis of active TB.

Active case finding Most patients with active TB present with symptoms that are investigated and a correct diagnosis eventually made. This is passive case finding. An important part of the work undertaken by TB services in the UK is active case finding where individuals or groups considered to be at high risk of TB, such as contacts of cases and new entrants from high prevalence countries, are screened.

Active case finding using mass mobile chest radiography was widely used until the early 1980s. At this time the incidence of TB in the UK was at its lowest and the service was not considered to be cost-effective or safe. However, the massive increase in incidence of TB, particularly in groups who find access to health care difficult (eg, the homeless), along with the development of safer digital x-ray technology (which uses lower radiation doses) has prompted a proposal to reinstate a mobile chest x-ray service in London.

Diagnosis of latent TB — TSTs

The TST is currently the only technique for diagnosing latent TB. A potential future test for latent TB is the ELISPOT assay (see Panel 2). The basic principle of the TST is that a protein prepared from a culture of MTB, tuberculin purified protein derivative (PPD), is injected into the skin. If the patient has been infected with MTB a local, delayed-type hypersensitivity reaction will occur within days. The resultant induration can be assessed. Two main methods of tuberculin skin testing exist: the Heaf test and the Mantoux test.

Heaf test The Heaf test is the more widely used in the UK. A small drop of tuberculin PPD (100,000u/ml) is placed on the skin, usually on the underside of the left forearm, and a Heaf gun (a six-pronged multiple puncture head) injects the PPD into the epidermis. The results are read at seven days

Panel 2: ELISPOT assay

A recently developed enzyme-linked immunospot (ELISPOT) assay has the potential to diagnose active and latent TB, but is not yet available in routine practice. ELISPOT is, in essence, a blood test that can detect a specific immune response to MTB overnight. An individual's immune response to infection with MTB includes sensitisation of his or her T-lymphocytes to proteins in the MTB bacillus. If these T-lymphocytes encounter the proteins again, for example, in a test tube, they will reactivate and release the cytokine interferon- γ , which can be detected. If the proteins to which the T-lymphocytes respond are unique to MTB (eg, the early secretory antigenic target-6 [ESAT-6] and culture filtrate protein 10 [CFP 10]), the test will be specific for and sensitive to MTB infection.

A positive Heaf test indicates exposure

and graded from 0 (no reaction) to 4 (solid induration over 100mm with or without ulceration). Grades 0 or 1 are negative, grade 2 is compatible with previous BCG vaccination (if appropriate) and grades 3 or 4 are positive.

Mantoux test The Mantoux test is used in the US and in paediatric practice in the UK. It is administered by using a needle and syringe to inject 5 tuberculin units, intradermally. The diameter of induration is measured at between 48 and 72 hours. Three cut-off levels for a positive test are recommended, depending on the patient's risk of developing TB. For instance, ≥ 5 mm is considered a positive result in a patient with HIV but, if the risk of TB is low, ≥ 15 mm induration is positive.

TSTs are used in three situations. First, they are used to diagnose latent TB in patients who would benefit from treatment or chemoprophylaxis (to prevent active disease). The risk of developing active TB in the presence of a positive TST varies. In recent contacts of a person with infectious TB it is estimated to be 13 cases per 1,000 person years. In people with HIV it is between 35 and 162 cases per 1,000 person years. Other conditions associated with increased risk include renal failure, diabetes and silicosis (lung disease caused by inhalation of silica dust). Transplant patients and others receiving immunosuppressive treatments are also at greater risk. British Thoracic Society guidelines for identifying latent TB in patients planned for therapy with anti-tumour necrosis factor α drugs are in development.

The second use is in order to offer TST-negative children a BCG vaccine if they are contacts of people with TB or as part of a national TB prevention strategy.

Thirdly, the TST can supply supplementary evidence in patients in whom the diagnosis of active TB is not clear (eg, where clinical samples for microbiological analysis are not available). However, most patients with a positive TST do not have active TB and, conversely, 10 to 25 per cent of patients with active TB have a negative TST result.

Action: practice points

Reading is only one way to undertake CPD and the Society will expect to see various approaches in a pharmacist's CPD portfolio.

1. Look out for the imminent guidelines for diagnosing latent tuberculosis infection in patients planned for anti-TNF α therapy.
2. Review the information on BCG vaccines in the British National Formulary.
3. Apart from the traditional procurement and supply role, consider what other roles pharmacists could play in tuberculosis care. Visit the Stop TB partnership website (www.stoptb.org)

Evaluate

For your work to be presented as CPD, you need to evaluate your reading and any other activities.

Answer the following questions:

What have you learnt?

How has it added value to your practice? (Have you applied this learning or had any feedback?)

What will you do now and how will this be achieved?

TST limitations TSTs can also give false positive results in patients who have had BCG or been infected with MOTTs. False negatives can occur in patients unable to mount an immune response such as in advanced HIV disease. Other limitations of the TST include inter-observer variability in reading results, patients not returning to have their TST read and interpretation of results in the context of risks for developing active TB.

Reference

1. Rose AMC, Watson JM, Graham C, Nunn AJ, Dribniewski F, Ormerod LP et al. Tuberculosis at the end of the 20th century in England and Wales: results of a national survey in 1998. *Thorax* 2001;56:173–9.

Further reading

- Joint Tuberculosis Committee of the British Thoracic Society. Control and prevention of tuberculosis in the United Kingdom: Code of Practice 2000. *Thorax* 2000;55:887–901.
- American Thoracic Society/Centers for Disease Control and Prevention. Targeted tuberculin testing and treatment of latent tuberculosis infection. *American Journal of Respiratory and Critical Care Medicine* 2000;161:S221–47.
- Lalvani A. Spotting latent infection: the path to better tuberculosis control. *Thorax* 2003;58:916–8.
- Drobniewski FA, Caws M, Gibson A, Young D. Modern laboratory diagnosis of tuberculosis. *The Lancet Infectious Diseases* 2003;3:141–7.
- Schluger NW. Changing approaches to the diagnosis of tuberculosis. *American Journal of Respiratory and Critical Care Medicine* 2001;164:2020–24.

Topics in this series

Further articles in this series will look at:

- Standard management of TB in the UK
- Prevention of TB and treatment of drug-resistant TB