Public Health England

Protecting and improving the nation's health

Molecular diagnosis of tuberculosis: Information for healthcare professionals

About Public Health England

Public Health England exists to protect and improve the nation's health and wellbeing, and reduce health inequalities. It does this through world-class science, knowledge and intelligence, advocacy, partnerships and the delivery of specialist public health services. PHE is an operationally autonomous executive agency of the Department of Health.

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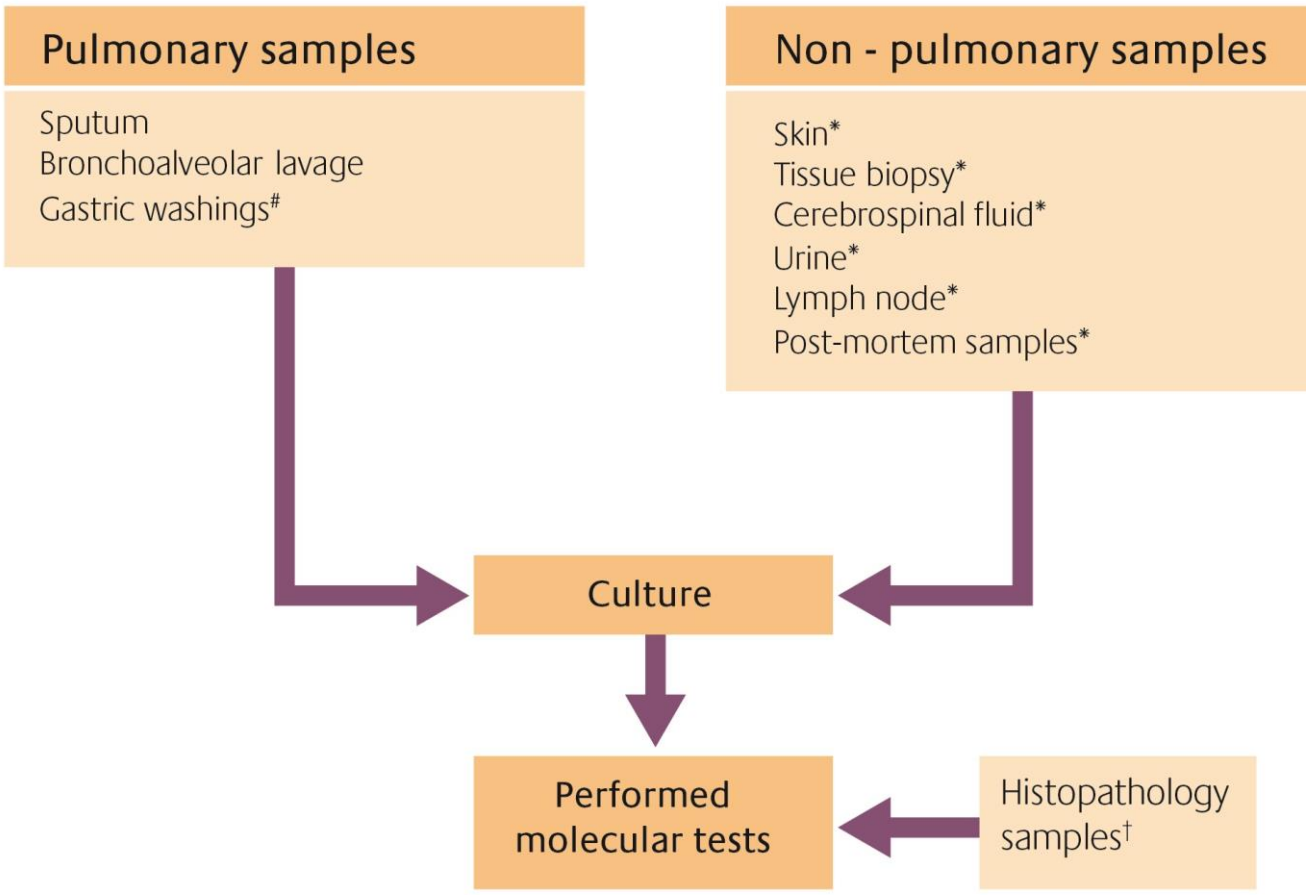
Introduction

This is a brief guide for healthcare staff investigating patients suspected of tuberculosis (TB). TB disease (often known as active TB, and usually associated with symptoms) may be diagnosed in a number of ways. Laboratory tests can either support or confirm clinical symptoms of TB.

The current standard is a positive mycobacterial culture from a body fluid or tissue (see Figures 1 and 2). This is a relatively slow process, often taking days to weeks for the organism to grow and then be specifically identified. Faster methods include smear staining and microscopy for acid fast bacilli. However, this is much less sensitive than culture, and molecular tests are now widely available that have increased sensitivity compared to smear and are also rapid.

This summary provides information on molecular tests, and their role in the diagnosis of active TB. While microbiology tests are important in the diagnosis of TB, they should always be considered in conjunction with the clinical presentation and imaging.

Figure 1. Samples required for microbiological diagnosis of TB

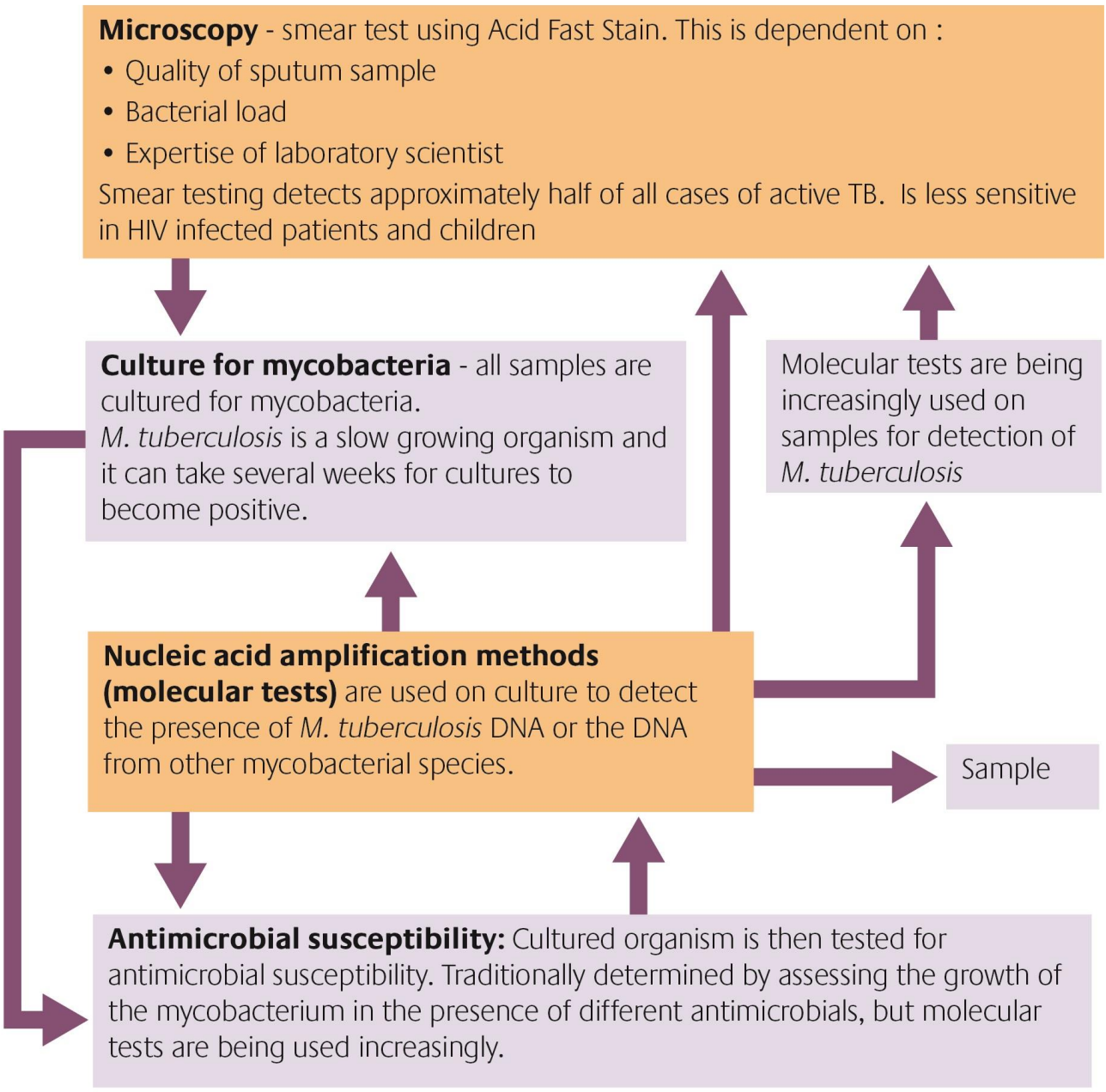


* Less likely to be used for molecular testing but in specific cases can be used after discussion with a microbiologist. Some commercial tests are only licensed for respiratory samples.

† May not always achieve amplification in a formalin fixed sample

Gastric washings (swallowed respiratory secretions) are discouraged except in children and are not useful for molecular tests

Figure 2. Microbiology tests available for the diagnosis of active TB



Molecular tests

In microbiology, the term molecular test is conventionally used to describe tests that detect pathogen nucleic acid (DNA or RNA). All molecular diagnostic tests have three stages: extraction, amplification and detection.

Extraction of nucleic acid

The extraction process should be carried out in a containment level 3 laboratories and will provide inactivation of organisms since Hazard Group 3 species may be present.

Amplification

The nucleic acid in the sample or culture is amplified so that it can be detected. Polymerase chain reaction (PCR) is an example of a method for amplification, but a variety of other methods are available.

Detection

Methods of detection of the amplified nucleic acid also vary and include the use of electrophoresis, line probe assays or real time detection. Depending on the format, some tests may take two days for results to be available whereas others may produce results in a few hours.

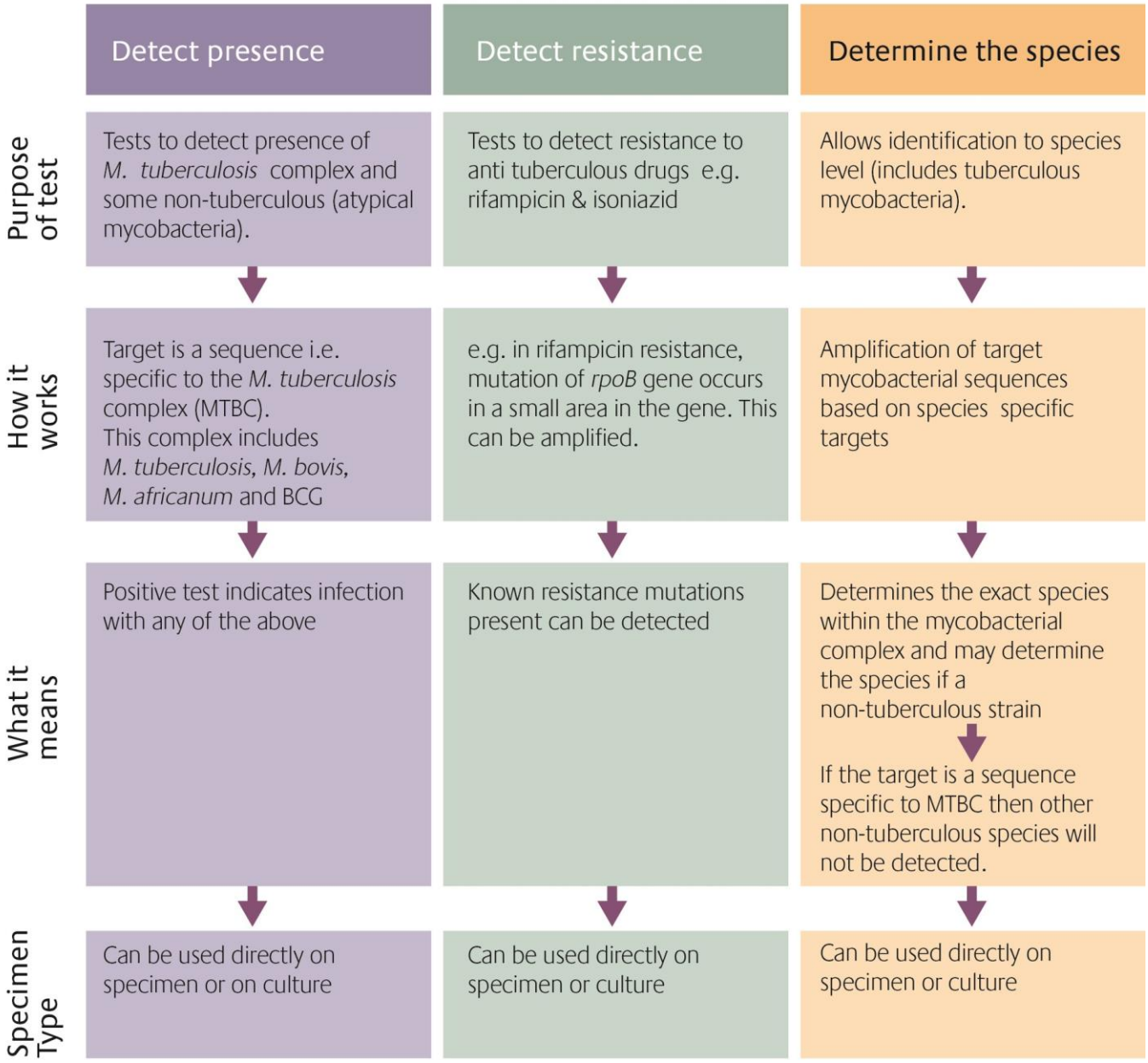
Different types of molecular testing technology are available for TB

Multiple commercial tests are available and new tests are also being developed. Some laboratories have developed local “in-house” tests. The choice of test depends on clinical need and local laboratory factors such as numbers of specimens received by the laboratory, access to molecular platforms and expertise.

Line probe assays and the Cepheid MTB/RIF test which is run on the GeneXpert platform are commercial systems that are recommended by the WHO, for resource poor countries – where financial support is available for the cost of tests. In developed countries this format of test is convenient but no more accurate or reliable than other test systems and may be more expensive.

This document does not consider individual assays and does not endorse any particular commercial assay.

Figure 3. Molecular testing



The provision of molecular tests for TB

Direct molecular tests on samples can be performed in a hospital laboratory or in reference laboratories. Hospital laboratories can provide these tests locally or send it to the Mycobacterial Reference Unit in London, Birmingham and Newcastle. The latest tests are very simple to use and do not need a full molecular laboratory. As the WHO has indicated, for rifampicin resistance (and where it is used as marker of MDRTB) the positive predictive value of the Cepheid test, for example, only exceeds 90% when the prevalence for resistance exceeds 15%, which is rare globally. In other words most tests that are positive for rifampicin resistance are false positives hence the need for a second confirmatory test (either another molecular test or a phenotypic drug resistance assay).

The decision whether to provide these tests on site or to use a reference laboratory is made at a local level. Where a local laboratory does not offer molecular testing directly on samples, samples may be referred to a reference laboratory after discussion with the local microbiologist.

Molecular test for identification of culture isolates are provided by the reference laboratories and by some larger hospital laboratories.

How local laboratories choose which molecular test to provide

This decision depends firstly on clinical need. However, there are many other considerations that determine which molecular tests (if any) are provided locally.

Laboratories may provide commercial tests or use an in-house method. In-house methods may be cheaper but have several disadvantages. These include variation in quality control, evaluation and validation, lack of a regulatory body approval and the requirement for a higher scientific input into assay design and evaluation. Any reputable laboratory must have conducted an evaluation of their in house methods before introducing it and should be able to supply this data on request. A global surveillance programme is difficult for in-house tests and also to make appropriate changes based on emerging sequence data.

Commercial assays are advantageous in that the manufacturer provides a complete package of reagents, equipment, training and support with quality assurance.

The different commercial assays vary in the molecular methods, equipment and the available repertoire of tests. Some commercial assays and most in-house tests are labour-intensive whereas others are almost fully automated with the option to run single samples rather than in batches. Some manufacturers propose that these tests can be performed in the near patient environment. There are also differences in cost between the different tests.

Individual laboratories will choose a test based on factors such as local epidemiology, level of molecular expertise in their staff, equipment availability, and price.

Laboratories that have a large TB workload are likely to offer molecular testing on-site whereas others will refer the work to other centres. Depending on the numbers of specimens some laboratories may offer a daily testing service, but most will run routine diagnostic samples in batches once or twice a week. Most reference centers offer daily services.

Molecular tests and influencing patient outcome

Early diagnosis of active TB can impact upon patient outcome and public health control of TB, and may ultimately provide cost savings.

Rapid results can result in prompt antibiotic treatment and isolation of patients, prevention of transmission, reduced hospital stay, and better targeted contact investigations.

Some molecular tests can also provide an early indicator of antimicrobial resistance, with presumed improvement in the early treatment and control of drug-resistant tuberculosis.

The cost-effectiveness of this approach is currently being investigated.

Molecular tests and information on antimicrobial susceptibility

The mechanisms by which *Mycobacterium tuberculosis* can develop resistance to antimicrobials have been determined for many of the different drugs in use. In many cases, resistance has developed as a result of mutations in specific genes for example *rpoB* in the case of rifampicin-resistance. In the case of *rpoB*, many of these mutations occur in a small area of the gene. This hotspot can be amplified and known resistance mutations present can be detected.

There are many commercial tests that can detect rifampicin-resistance using this method and some that can detect mutations in other drugs including isoniazid, ethambutol, quinolones, amikacin, kanamicin and capreomycin.

These tests can be performed directly on patient specimens or on cultures. Consequently these results will be available much earlier than by conventional antimicrobial susceptibility methods that require a positive culture.

Any specimen or culture that requires molecular testing for drug-resistance should have results confirmed by conventional culture-based methods. This is for two main reasons:

- not all mutations that confer resistance have been determined and therefore cannot be tested by using the molecular method. Thus a strain of *M. tuberculosis* may be resistant to drug as a result of a mutation outside of the area examined by the assay or by some other as yet unknown mechanism
- some mutations do not always correspond to complete resistance and the drug can still be used, sometimes at a higher dose, eg quinolones

This is an area of current research. With current rapid technological developments, it is now possible to sequence the entire genome of bacteria very quickly. Comparison of the sequences of drug-resistant and sensitive strains may lead to discovery of further mutations associated with resistance in the future.

Other advantages of using molecular testing

The principle advantage of molecular testing is speed in obtaining a result, whether testing directly on samples or on cultured isolates.

In some situations molecular testing may provide information when conventional tests are negative, eg with smear negative samples.

Occasionally specimens from patients may not have been sent for microscopy and culture and a presumptive diagnosis has been made from histology of fixed tissue specimens.

Molecular tests on formalin fixed specimens are not always successful and are only used when fresh tissue is not sent to the microbiology laboratory.

Molecular typing methods produce more discriminatory results than older methods, are almost as sensitive as microbiological culture in liquid media and provide a powerful epidemiological tool.

The risks of false positive or false negative results

Molecular testing performed directly on samples for the detection of *M. tuberculosis* has variable performance depending on the specific test used. Generally these tests are only reliable if the quantity of *M. tuberculosis* present in the specimen is sufficient. For many tests, the threshold is similar to the number required for visualisation by microscopy. Some assays are more sensitive and can detect a proportion of smear-negative infections; however at present no molecular test performed directly on a sample is as sensitive as culture.

A recent international study of one of the more widely used tests showed an overall specificity of 99%, with a sensitivity of 98.3% in sputum smear positive TB patients and 76.9% in smear negative cases, compared to culture. This means that the rate of false positive results was very low in the study. The rate of false negatives in smear-positive patients was also very low, but about 1 in 4 smear-negative patients with culture

positive TB would test negative. There are more false negative results in HIV-infected patients. Overall the test detected 90.7% of culture positive TB.

However, the performance of any test also depends on the prevalence of the disease in the population being tested. Using this same test, in a population with a TB prevalence of 1%, which is higher than most populations in the UK, testing 1000 patients would lead to just 0.9 false negatives, but 9.9 false positives. For this reason, in low burden settings, the WHO recommends using these tests in conjunction with other information such as chest X-ray result.

Clinicians are good at detecting false positives for TB itself but cannot detect false positive drug resistance (see above).

Molecular testing and traditional methods

Molecular tests performed directly on samples can indicate the presence of *M. tuberculosis* and the presence of some genetic markers of drug resistance, particularly for rifampicin resistance. However, it is important that smear and culture are also performed. Smear tests will identify the presence of non-tuberculous (atypical) mycobacteria that are not detected by the molecular test for TB.

Culture provides additional drug resistance results, some of which cannot be detected by molecular testing. In addition, only culture can determine whether there are viable organisms present, since molecular tests will detect non-viable organisms even after successful treatment. In addition, the culture isolate is used for strain typing to inform public health interventions such as contact tracing.

Cost implications of molecular testing

Direct molecular testing on a sample is currently significantly more expensive than a sputum smear. The cost may be charged to the TB or microbiology service depending on local arrangements.

Molecular tests that enable rapid detection of TB may theoretically lead to a reduction in overall TB healthcare costs, for example by reducing number of follow up clinic visits. However, there is no evidence on whether this is the case. An evaluation of the cost-effectiveness of TB molecular tests in the NHS is currently under way.

Use of the test in patients undergoing treatment or following treatment

Molecular tests may sometimes be useful in patients undergoing treatment. A molecular test may remain positive even when mycobacteria present in a specimen are non-viable and cannot be cultured. This may be helpful if a patient has started treatment before specimens were obtained.

They may also be used for rapid detection of drug resistance which can evolve in patients on treatment. This is much faster than detecting resistance by culture and allows treatment to be changed quickly.

Molecular tests are not useful following treatment as a test of cure. As already described, they can detect dead organisms and so a positive test does not indicate treatment failure. A negative molecular test after treatment does not prove treatment has been successful, as none of the tests are 100% sensitive, particularly with a low bacterial load.

Interpreting the results of molecular tests

Interpretation of molecular TB microbiology results is not straightforward and does require specialist input. This may be from a microbiologist, respiratory physician or infectious diseases physician, depending on expertise and local arrangements. The increasing use of molecular tests makes education of laboratory staff, clinicians and healthcare providers important.

In centres where molecular TB testing is not provided locally, there may not be sufficient expertise to work with the results of these tests. Expert microbiological advice is available through the PHE reference laboratories.

Molecular tests and typing of *M. tuberculosis*

Molecular tests can be used to type and compare strains of *M. tuberculosis*. This is useful for epidemiology, eg for establishing transmission between contacts. In England, all *M. tuberculosis* isolates are typed under the National TB Strain Typing initiative to provide national and local epidemiological information. This is done using a molecular test called MIRU-VNTR (mycobacterial interspersed repeat units – variable number tandem repeats).

The *M. tuberculosis* genome has a number of areas where there are repeat sequences in the DNA. This test counts the repeats at 24 places on the *M. tuberculosis* genome and uses this to produce a 24-digit code which is the strain type designation. This is a highly discriminatory method. If two unrelated strains are randomly sampled, only in 0.02% of instances will they have the same typing result.