Micropathology Ltd
Tel 24hrs: +44 (0) 24 - 76 – 323222
Fax / Ans: +44 (0) 24 - 76 - 323333
University of Warwick Science Park, Venture Centre, Sir William Lyons Road, Coventry CV4 7EZ
Website: www.micropathology.com E-mail: info@micropathology.com

Mycobacterium tuberculosis Complex*:* Rifampicin Resistance

Tuberculosis (TB) is an airborne infectious disease caused by species of the *Mycobacterium tuberculosis* complex. Ranked as the ninth cause of death globally, TB remains a persistent public health threat worldwide and is considered the leading cause of mortality by a single infectious agent, particularly in developing and low-income countries(1).

TB is a chronic disease with high mortality and morbidity, and can be latent or active. Latent TB is asymptomatic and non-contagious, and has high prevalence rates of ~51.5% and ~28.1% in low/middle-income countries and high-income countries, respectively(2). Generally, without treatment, ~5-10% of individuals infected with species of the *M. tuberculosis* complex will develop active TB in their lifetime, with 50% developing active tuberculosis within two years of initial infection(3). Individuals who are positive for Human Immunodeficiency Virus (HIV) and the immunocompromised have an increased risk of developing active TB(3).

TB is endemic to certain regions of the world, such as Africa, Russia, Eastern Europe, Asia, Latin America and the Caribbean, and before the use of antibiotics, it was a major health problem in the UK(4). Incidence has since declined, although cases have gradually increased in recent years, particularly amongst ethnic minority communities who originate from countries with a high prevalence of TB. Social risk factors such as imprisonment, alcohol and drug misuse and homelessness are also associated with contracting TB.

To reduce the risk of drug-resistance developing, a combination of antibiotics, typically over a six-month duration, are used for the treatment of TB. Standard treatment comprises four first-line antibiotics: rifampicin, isoniazid, pyrazinamide and ethambutol. Individuals with pulmonary TB may remain contagious until approximately two to three weeks into the course of treatment.

Since the 1990s the ability to manage TB globally has been challenged by the emergence of drug-resistant strains within the *Mycobacterium tuberculosis* complex. Of greatest concern are multi-drug resistant (MDR) strains, defined as those resistant to two of the most effective first-line anti-TB agents *in vitro*: isoniazid and rifampicin. MDR-TB is problematic, as it requires the use of alternative drugs which can have more adverse side effects. The development of MDR-TB is associated with poor drug compliance and social factors including poverty, homelessness, or recreational drug use.

In the UK it is recommended that rifampicin resistance testing should be performed on suitable samples from a patient where TB is clinically suspected and also from those where one or more significant risk factor for MDR-TB is identified[5,6]. Such risk factors include a history of previous TB drug treatment, particularly if poor treatment adherence and compliance is known; contact with a known case of MDR-TB and birth or residence in a country in which a high proportion (5% or more) of new TB cases are multidrug-resistant as reported by the World Health Organisation[7].

In 2021, 10.6 million new cases of TB were identified world-wide; of these an estimated 450,000 had resistance to rifampicin (RIF), which requires rapid, accurate detection and characterization to initiate appropriate alternative treatment regimens[5].

Resistance is mediated exclusively by chromosomal mutations that affect either the drug target itself or bacterial enzymes that activate prodrugs. A large number of mutations have been identified in the *rpoB* gene (encoding the β subunit of RNA polymerase) of *M. tuberculosis* that confer rifampicin resistance and so serve as a surrogate marker for MDR-TB. A region of 81 nucleotides, spanning codons 507-533 of the *rpoB* gene, described as the rifampicin-resistance-determining region (RRDR), detects more than 96% of rifampicin-resistant strains within the *M. tuberculosis* complex[8,9].

Our assay:

At Micropathology Ltd, our semi-nested PCR assay amplifies a region of the *rpoB* gene (encoding the β-subunit of RNA polymerase) in species of the *Mycobacterium tuberculosis* complex. As mutations within the rifampicin resistance determining region (RRDR) of the *rpoB* gene have been associated with more than 96% of rifampicin resistant strains of the MTBC, our assay involves analysis of sequence data from the RRDR to identify rifampicin resistance mutations.

From Feb 2025, sequence analysis and interpretation are based upon the WHO 2023 report, ‘Catalogue of mutations in *Mycobacterium tuberculosis* complex and their association with drug resistance, 2nd ed.’ Mutations described are now according to *Mycobacterium tuberculosis* complex numbering, replacing the previously used *Escherichia coli* nomenclature.

This assay is validated for testing samples positive for *M. tuberculosis* complex DNA.

Turnaround times are stated in the user manual with results usually available in practice much sooner than the given time frame. Where there is a delay, we are usually confirming a result and addressing clinical data given with the specimen.

References:

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Useful links:

Policy paper; Tuberculosis (TB): action plan for England, 2021 to 2026 (UKHSA) Tuberculosis (TB): action plan for England, 2021 to 2026 - GOV.UK (www.gov.uk)

TB diagnosis, microbiology and drug resistance in England, 2021 (UKHSA) TB diagnosis, microbiology and drug resistance in England, 2021 - GOV.UK (www.gov.uk)