



***Mycobacterium tuberculosis* 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 2 years of initial infection⁽³⁾. Individuals who are positive for 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, usually over a 6 month duration, are used for the treatment of TB. Standard treatment for TB comprises four first-line antibiotics: rifampicin, isoniazid, pyrazinamide and ethambutol. Individuals with pulmonary TB may remain contagious until approximately 2 to 3 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 of 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, 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.

TB drug resistance should be considered if patients have the following risk factors: 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 Organization⁽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⁽⁶⁾.

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 *Mycobacterium tuberculosis* complex, our assay involves analysis of sequence data from the RRDR to identify rifampicin resistance mutations within species of the *Mycobacterium tuberculosis* complex.

Currently, UKAS accredited specimen types for this assay include: sputum, tissue, fixed tissue, CSF, BAL and cultures.

Turnaround times are stated in the user manual (<http://www.micropathology.com/customer-downloads-handbooks.php>) 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:

- (1) Miggiano, Riccardo, et al. "Mycobacterium Tuberculosis Pathogenesis, Infection Prevention and Treatment." *Pathogens*, vol. 9, no. 5, 2020, p. 385., <https://doi.org/10.3390/pathogens9050385>.
- (2) Lee, Seung Heon. "Tuberculosis Infection and Latent Tuberculosis." *Tuberculosis and Respiratory Diseases*, vol. 79, no. 4, 2016, p. 201., <https://doi.org/10.4046/trd.2016.79.4.201>.
- (3) Park, S.Y., Han, S., Kim, YM. et al. Risk of active tuberculosis development in contacts exposed to infectious tuberculosis in congregate settings in Korea. *Sci Rep* 10, 1306 (2020). <https://doi.org/10.1038/s41598-020-57697-1>
- (4) Kyu, Hmwe Hmwe, et al. "Global, Regional, and National Burden of Tuberculosis, 1990–2016: Results from the Global Burden of Diseases, Injuries, and Risk Factors 2016 Study." *The Lancet Infectious Diseases*, vol. 18, no. 12, Dec. 2018, pp. 1329–1349., [https://doi.org/10.1016/s1473-3099\(18\)30625-x](https://doi.org/10.1016/s1473-3099(18)30625-x).
- (5) Pai, Madhukar, et al. "Tuberculosis." *Nature Reviews Disease Primers*, vol. 2, no. 1, 2016, <https://doi.org/10.1038/nrdp.2016.76>.
- (6) Van Der Zanden, A. G., et al. "Use of DNA Extracts from Ziehl-Neelsen-Stained Slides for Molecular Detection of Rifampin Resistance and Spoligotyping of *Mycobacterium Tuberculosis*." *Journal of Clinical Microbiology*, vol. 41, no. 3, 2003, pp. 1101–1108., <https://doi.org/10.1128/jcm.41.3.1101-1108.2003>.