Whitepaper

Hain Lifescience - a Bruker Company

Mycobacteria testing in general laboratories

Introduction

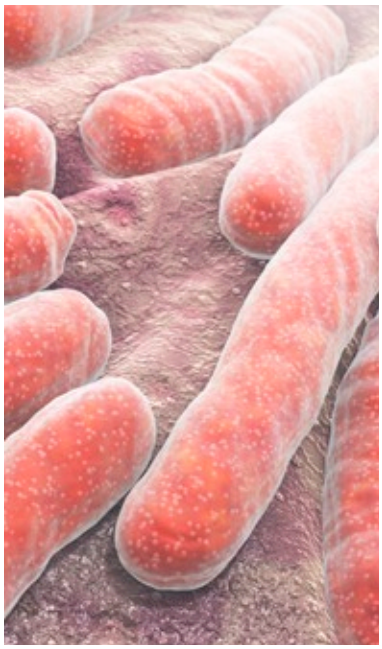
Mycobacterium – a genus of the family *Actinobacteriaceae* – comprises more than 190 validated species[1], including pathogens known to cause serious diseases in humans, such as tuberculosis (TB), (MTBC - *Mycobacterium tuberculosis* complex) and leprosy (*Mycobacterium leprae*)[2]. Mycobacteria are immobile, slow- or rapid-growing, rod-shaped, Gram-positive bacteria. Due to their special cell wall characteristics they are called acid-fast and cannot be stained by the Gram stain procedure, but with specific other stainings such as Auramine/Rhodamine or Ziehl-Neelsen.

Mycobacteria can be divided into three groups:

- *Mycobacterium tuberculosis* complex – causative pathogens of tuberculosis
- *Nontuberculous mycobacteria* (NTM) – with different pathogenic potential
- *Mycobacterium leprae* – causative pathogen of leprosy

Mycobacteria are widespread organisms, typically living in soil, water, animal tissue and food sources. Mycobacteria can colonize their hosts without inducing any pathogenic signs or can cause latent infections which can lead to severe diseases.

The infections resulting from MTBC are difficult to treat because of some intrinsic resistances against antibiotics like penicillin G, sulfonamides, tetracycline, erythromycin, and chloramphenicol[3], and NTM are even harder to treat. Mycobacteria can survive exposure to acids, alkalis, detergents or oxidative bursts. Fortunately, most NTM are susceptible to the antibiotics clarithromycin and rifamycin, although antibiotic resistant strains have also emerged, presenting a new challenge for global healthcare bodies.



Latest status of the TB epidemic

In 1882, the German physician Robert Koch discovered *M. tuberculosis* to be the causative pathogen of pulmonary TB, which was followed by an improvement in diagnostics in the following decades. TB pathogens are transmitted via droplet infection through the air by coughing, sneezing and speaking. The risk of infection is greatly increased in high incidence countries with densely populated areas.

TB is one of the top 10 causes of death throughout the world and is the leading cause of death from a single infectious agent (above HIV/AIDS and malaria). In 2017, it was estimated that 10 million people per year developed the disease.[4] Two thirds of these cases occurred in just eight countries: India (27%), China (9%), Indonesia (8%), the Philippines (6%), Pakistan (5%), Nigeria (4%), Bangladesh (4%) and South Africa (3%). About 1.7 billion people (23% of the world's population) are estimated to have a latent TB infection and are therefore at risk of developing active TB disease during their lifetime.[5]

Millions of deaths every year are prevented by the successful early diagnosis followed by proper treatment of TB. However, drug-resistant TB continues to be a public health crisis. The recent estimates demonstrate that, in 2017, 558,000 people developed TB with resistance to rifampicin (a rifamycin derivative) (RR-TB), the most effective first-line drug. Additionally, 82% of these were resistant to the second first line drug, isoniazid, and are therefore described as multidrug-resistant TB (MDR-TB).[5] [6] Treatment success remains low (around 55% globally) and closing the gap between detection and treatment requires more enhanced and specific drug susceptibility testing among those diagnosed with TB. The therapy for MDR TB is more time-consuming and is characterized by more frequent and severe side effects. This has led to a lower therapy compliance rate among the treated patients and therefore resulted in further increase of the number of drug resistances.

According to a World Health Organization (WHO) report, extensively drug resistant (XDR)-TB is defined as MDR-TB plus resistance to at least one drug in both of the two important classes of medicines in an MDR-TB regimen: fluoroquinolones and second-line injectable agents (amikacin, capreomycin or kanamycin).[7]

Identifying the drug resistance pattern is therefore crucial to establishing a successful treatment plan to cure the patient and consequently stop the transmission of resistant strains.

Benefits of GenoType CM*direct*

- Differentiation between NTM and *M. tuberculosis* complex
- 27 clinically relevant NTM detected simultaneously
- Direct detection on sputum specimens as starting material
- High reliability with internal controls
- Time saving: results within 5 hours only
- User-friendly: a ready-to-use amplification mix
- Versatile: automated or manual processing, one platform for several tests

Benefits of FluoroType® MTBDR

- Novel FluoroType® technology based on asymmetric excess PCR and detection via Lights-On and Lights-Off probes
- Reliable MDR-TB diagnostics within 3 hours only
- Resistances to both first-line drugs, rifampicin and isoniazid, as well as mono-resistances are detected
- Flexible, user-friendly and intelligent Fluoro-Software® for evaluation and result interpretation
- Common resistance-mediating mutations are specified
- In addition, rare or so far unknown mutations in the target genes are also shown

NTM and its effect on humans

Most NTM species are non-pathogenic and transmission from human to human is unclear. Some NTM species, however, can be harmful to people, particularly those who are immunocompromised or suffer from previous pulmonary or other diseases. Clinical presentation of infections from NTM include predominantly pulmonary infection, disseminated infection, skin disease and lymphadenitis.

NTM are currently classified according to their growth rate – slow-growing mycobacteria (SGM) requiring more than seven days to grow, and rapid-growing mycobacteria (RGM), requiring less than seven days to grow under ideal conditions. There are only few standard therapy recommendations for NTM infections. Detection of the species with reliable resistance testing provides the only basis for successful treatment. In the last few decades, the number of NTM infections observed worldwide seemed to be increased, especially in countries that have low TB prevalence.

The difficulty of testing for mycobacteria in the general lab

The distinction between TB and NTM is essential for diagnosis and treatment, and the course of action depends on the respective mycobacterial species. Genotypic (molecular) methods for species differentiation and resistance testing are valuable tools in TB and NTM diagnostics and offer considerable advantages compared with time-consuming conventional methods such as biochemical testing and conventional phenotypic resistance testing.

The process for identifying TB using traditional culture methods takes regularly between 2-4 weeks for a positive result, and between 6-8 weeks for a negative result (which then requires further testing) depending on whether solid or liquid media have been used. This is considerably slower compared with genetic testing, where identification for TB and NTM can be done in under 3 hours. Genetic testing offers a clear advantage for patients, since resistance patterns are earlier available for adequate treatment.

Furthermore, there is no requirement for a laboratory to have biosafety level 3 laboratory (BSL3) credentials for molecular methods, because these tests can be performed from direct samples. Via the direct sampling method, any laboratory is able to run genetic testing on samples to determine the presence or absence of *M. tuberculosis* and conduct further testing to identify NTM.

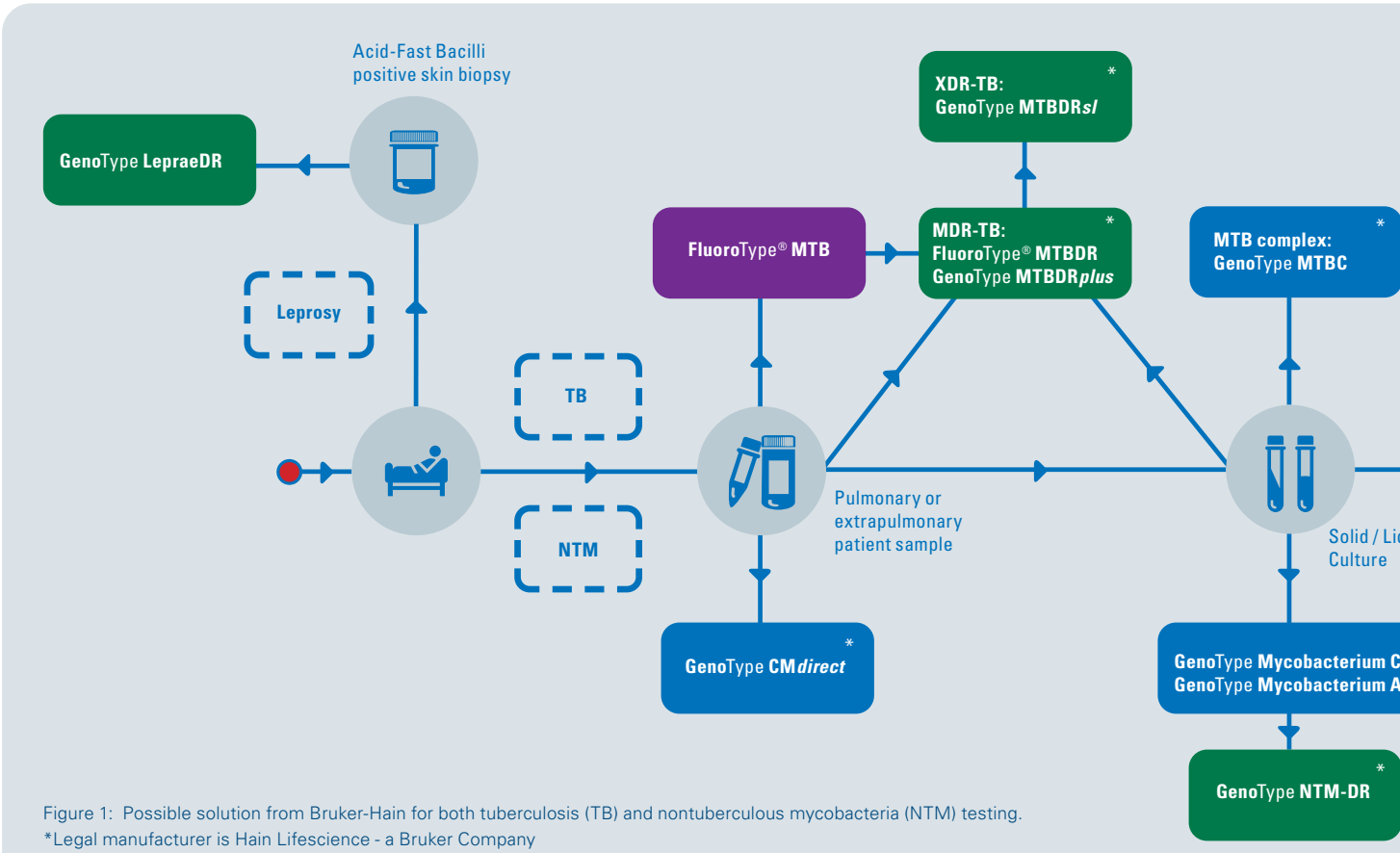
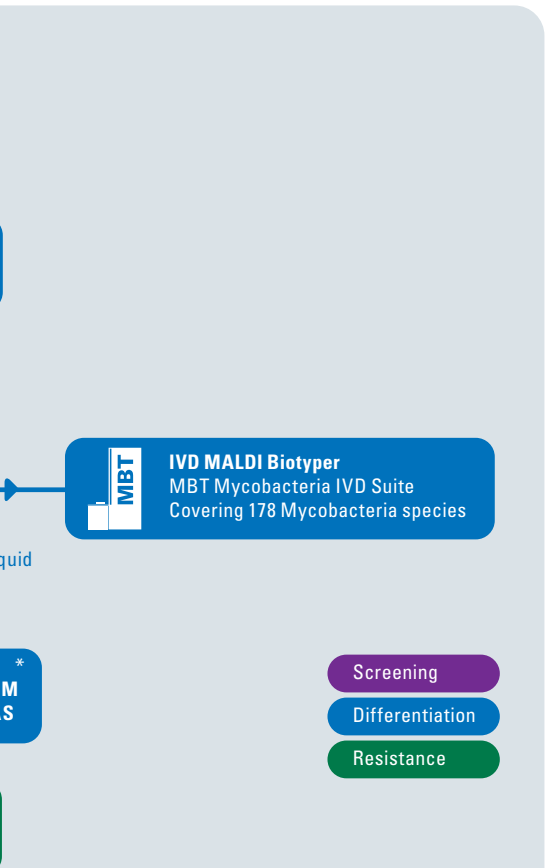


Figure 1: Possible solution from Bruker-Hain for both tuberculosis (TB) and nontuberculous mycobacteria (NTM) testing.
*Legal manufacturer is Hain Lifescience - a Bruker Company

In May 2016, the WHO issued new recommendations on the use of a rapid diagnostic test – the line probe assay (LPA) GenoType MTBDR*sl* – to detect resistance to second-line anti-TB drugs (SL-LPA)[8]. The test is the most reliable method of ruling out resistance to the second-line antibiotics fluoroquinolones and the injectable drugs capreomycin, kanamycin and amikacin, enabling clinicians to set patients on the proper regime at the earliest time[8].

Detection of any second-line resistance by the SL-LPA means that MDR-TB patients should not be enrolled on the shorter regimen, as this could jeopardize their treatment outcome and fuel the development of XDR-TB. Patients detected with XDR-TB should also not be enrolled on the shorter regimen, but require carefully designed individual regimens to optimize successful treatment.



Clinical impact: Case examples

Professor Robert Warren, Unit Director and Chief Specialist Scientist, South African Medical Research Council (SAMRC), has published more than 280 papers in the field of TB. Prof. Warren's research is largely based on molecular epidemiology and he has continually challenged assumptions related to TB with this work. His findings have demonstrated that transmission of TB occurs largely outside of the household and that the drug resistance epidemic is driven by transmission, especially in previously treated patients, implying reinfection.

South Africa is considered the largest consumer of molecular diagnostic testing for TB, and in Cape Town alone, more than 15,000 line-probe assays are estimated to be carried out per year. Prof. Warren's experience with this type of testing is extensive.

"The picture in South Africa is very different in terms of the incidence of TB," explains Prof. Warren, continuing: *"The country already had a high incidence rate but with the onset of HIV in the 1980s, this rapidly accelerated until our rates were among the highest in the world. The focus of our health system in the early 2000s was to halt the increase of drug-susceptible TB, but the downside of this is that drug-resistant TB got overlooked. Our figures now show that we appear to be on the downward trend for susceptible TB incidence, but now the focus needs to shift onto drug-resistant TB."*

The molecular tools that were developed by Hain Lifescience changed the landscape for testing in South Africa. Convincing physicians to move away from the traditional phenotypic testing to genetic testing was an initial barrier to the introduction of this method but once the benefits became clear the adoption was swift.

Prof. Warren describes his role in developing TB identification methods: *"At the research center, we have been responsible for evaluating the diagnostic tools that are available on the market before passing our findings to an independent panel, who then make recommendations to the Department of Health. From the work that we have carried out, the line-probe assay is now one of our most used testing methods for second-line TB identification."*

"The future for molecular testing in the area of TB, in my opinion, will need to focus on the low to middle income countries that don't have the necessary infrastructure in place for complex or specialized testing", explains Prof. Warren. "There are two different schools of thought here – to develop a test that will deliver results against a defined set of drugs, or to develop a test that will provide results against a comprehensive set of drugs. Each solution has its place but either way, the test needs to be as simple as possible and the results easy for a lab technician to understand without specialist knowledge."

MALDI Biotyper® sirius system

Although incidence of positive identification of NTM is still relatively low in Switzerland (8.1 cases per 100,000 in 2018), the number is growing. The diagnostic laboratory at Lucerne Cantonal Hospital not only carries out testing and identification for the 800+ patients in the hospital, it takes in testing from other hospitals in the area. The laboratory can carry out up to 4,200 mycobacteria identification tests each year.

"The Bruker-Hain diagnostic tools have really helped to improve the identification rates," comments Dr. Wittwer. *"Using the GenoType NTM-DR, which allows for simultaneous detection and differentiation of NTM, the lab is now able to identify between 88.4% and 96% of all NTM strains. When used in conjunction with the MALDI Biotyper library from Bruker, the job of identification becomes even easier."*

"Our laboratory first carried out studies in 2013 to demonstrate the clinical efficacy of the Hain diagnostic tool in the area of NTM identification. The reduced time to identification (from 6-8 weeks down to only 1-2 days) has greatly improved the ability of the lab to provide clinically relevant information to the physicians in order to be able to improve patient outcomes."
Franziska Wittwer – Biomedical Analyst, Specialist Mycobacteriology, Lucerne Cantonal Hospital, Switzerland - NTM

The future of TB and NTM testing

The ability to optimize patient treatment by providing a rapid, reliable mycobacteria identification is improving health outcomes of TB and NTM infections worldwide. The future of TB and NTM testing is changing. As higher rates of TB are being detected in undeveloped countries where investment in laboratories and detection infrastructure is lower, the methods of testing need to be adjusted accordingly. There will be fewer trained technicians in these locations and molecular testing will need to become more intuitive to meet this throughput requirement.

The WHO recommendation of the Bruker-Hain SL-LPA molecular test to detect resistance to second-line anti-TB drugs indicates a step-change in the treatment of this disease. For these diagnostic tests to be applicable into the future, a more comprehensive outlook is required for assay development. The new innovative FluoroType® MTBDR and LiquidArray® technology from Bruker-Hain is leading the way in simplifying molecular tests and data interpretation protocols to improve their reliability. FluoroType® MTBDR is the first assay based on this technology and leads to results only previously achievable with sequencing. It can detect TB and more than 60 mutations in TB genes that result in 522 resistance patterns, providing relevant information to guide therapy. Based on this technology, further and more comprehensive assays are currently in development.

In its 'End TB Strategy', the WHO has a shared vision of a world free of TB with zero deaths, disease and suffering by 2035.[5] This requires a 95% reduction in the absolute number of TB deaths and a 90% reduction in incidence rate compared with the 2015 baseline. Early diagnosis of TB, including universal drug-susceptibility testing and systematic screening of high-risk groups, will be a key component in achieving this target.

For more information on the range of solutions available for rapid detection of TB and NTM, please visit:
https://www.hain-lifescience.de/en/products/microbiology/mycobacteria.html

References

1. King HC, Khera-Butler T, James P, Oakley BB, Erenso G, Aseffa A, Knight R, Wellington EM, Courtenay O (2017) Environmental reservoirs of pathogenic mycobacteria across the Ethiopian biogeographical landscape. PLoS One 12(3):e0173811. doi: 10.1371/journal.pone.0173811
2. Ryan KJ, Ray CG, eds. (2004). Sherris Medical Microbiology (4th ed.). McGraw Hill. ISBN 0-8385-8529-9.
3. Jarlier V and Nikaido H (1994) Mycobacterial cell wall: structure and role in natural resistance to antibiotics. FEMS Microbiol. Lett. 123:11–18.
4. Porvaznik I, Solovič I, Mokrý J (2017) Non-Tuberculous Mycobacteria: Classification, Diagnostics, and Therapy. Adv Exp Med Biol, 944: 19-25.
5. Tuberculosis (TB) – Data & Statistics, Centers for Disease Prevention and Control (CDC), 2018, https://www.cdc.gov/tb/statistics/default.htm.
6. TB Elimination: Extensively Drug-Resistant Tuberculosis (XRD TB), Fact Sheet, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention (CDC), 2013, https://www.cdc.gov/tb/publications/factsheets/drtb/xdrtb.pdf.
7. World Health Organization Global Tuberculosis Report 2018: https://www.who.int/tb/publications/global_report/en/
8. The use of molecular line probe assays for the detection of resistance to isoniazid and rifampicin, Policy Update, World Health Organization, 2016 https://apps.who.int/iris/bitstream/handle/10665/250586/9789241511261-eng.pdf?sequence=1.

About Bruker Corporation (NASDAQ: BRKR)

Bruker is enabling scientists to make breakthrough discoveries and develop new applications that improve the quality of human life. Bruker's high-performance scientific instruments and high-value analytical and diagnostic solutions enable scientists to explore life and materials at molecular, cellular and microscopic levels. In close cooperation with our customers, Bruker is enabling innovation, improved productivity and customer success in life science molecular research, in applied and pharma applications, in microscopy and nanoanalysis, and in industrial applications, as well as in cell biology, preclinical imaging, clinical phenomics and proteomics research and clinical microbiology. For more information, please visit: www.bruker.com.

Please contact your local representative for availability in your country.
Not for sale in the USA.

CE IVD

As of May 2021, Bruker Daltonik GmbH is now Bruker Daltonics GmbH & Co. KG.

Bruker Daltonics GmbH & Co. KG

Bremen · Germany
Phone +49 (0) 421-2205-0

Hain Lifescience GmbH

Nehren · Germany
Phone +49 (0) 7473-9451-0

info.md@bruker.com - www.bruker.com/microbiology