IVD

Expert Insights

- Improving Tuberculosis treatment with fast diagnosis and resistance detection

Mycobacteria

Innovation with Integrity

Improving Tuberculosis treatment with fast diagnosis and resistance detection

Researchers at the South African Medical Research Council (SAMRC) Centre for Tuberculosis Research at Stellenbosch University have evaluated the FluoroType® MTBDR VER 2.0* assay compared to the GenoType MTBDR*plus* VER 2.0* assay for tuberculosis detection and molecular drug susceptibility testing.

Working with Bruker

Prof. Robin M. Warren is Unit Director at the SAMRC's flagship research Centre for Tuberculosis Research housed within the Division of Molecular Biology and Human Genetics at Stellenbosch University in Cape Town, South Africa. Prof. Warren and his team collaborate with Hain Lifescience GmbH – a Bruker company – by evaluating the diagnostic accuracy of the FluoroType® MTBDR VER 2.0 assay for the detection of TB and first-line drug-resistant (DR) TB.

"I think the benefit of working with Bruker Molecular Diagnostics is multi-fold. The assays themselves have changed the landscape of how drug resistance and the diagnosis of TB is done in South Africa and globally. By providing rapid results and diagnosing patients early, the FluoroType® assays are potentially preventing ongoing transmission."

Tackling the Tuberculosis crisis

Tuberculosis (TB) remains one of the world's most deadly infectious diseases, contracted by around 10 million people in 2020, with more than a million cases resulting in death.[1] TB is caused by a bacterium called *Mycobacterium tuberculosis* (*M. tuberculosis*), which mainly attacks the lungs. Rapid and targeted antibiotic treatments are required to cure patients; therefore, a precise diagnostic testing method is essential.

Drug-susceptible TB is treatable and curable with a six-months course of first-line antimicrobial drugs including isoniazid (INH) and rifampicin (RIF). Long courses of treatment and side-effects caused by the drugs however, mean that patient adherence for the whole course of treatment is low. If anti-TB drugs are used inappropriately, through incorrect prescription or prematurely ending treatment, drug resistance can emerge in TB patients.

However, if *M. tuberculosis* doesn't respond to the most effective first-line drugs INH and RIF, second-line drugs need to be prescribed. These options are often accompanied by severe adverse events and can be costly and time-consuming.[2] How challenging these treatments are for multi-drug-resistant (MDR)-TB outlines the global success rate which, in 2018, was 59%.[2]

In addition, there is a global need for new and more efficient treatment regimens for both drug-susceptible and DR-TB. Many of the drugs used in treatments today cause nausea and other side effects, which, combined with long courses of treatment, means patients are more likely to stop mid-way through their treatment.

To reach the United Nations' Sustainable Development Goal to end the TB epidemic by 2030, appropriate treatments for MDR-TB and extensively drug-resistant (XDR)-TB are essential.[2] These should begin with rapid diagnosis for the prevention of significant morbidity, mortality, and further transmission of the disease.

The role of the SAMRC Centre in TB research and diagnosis

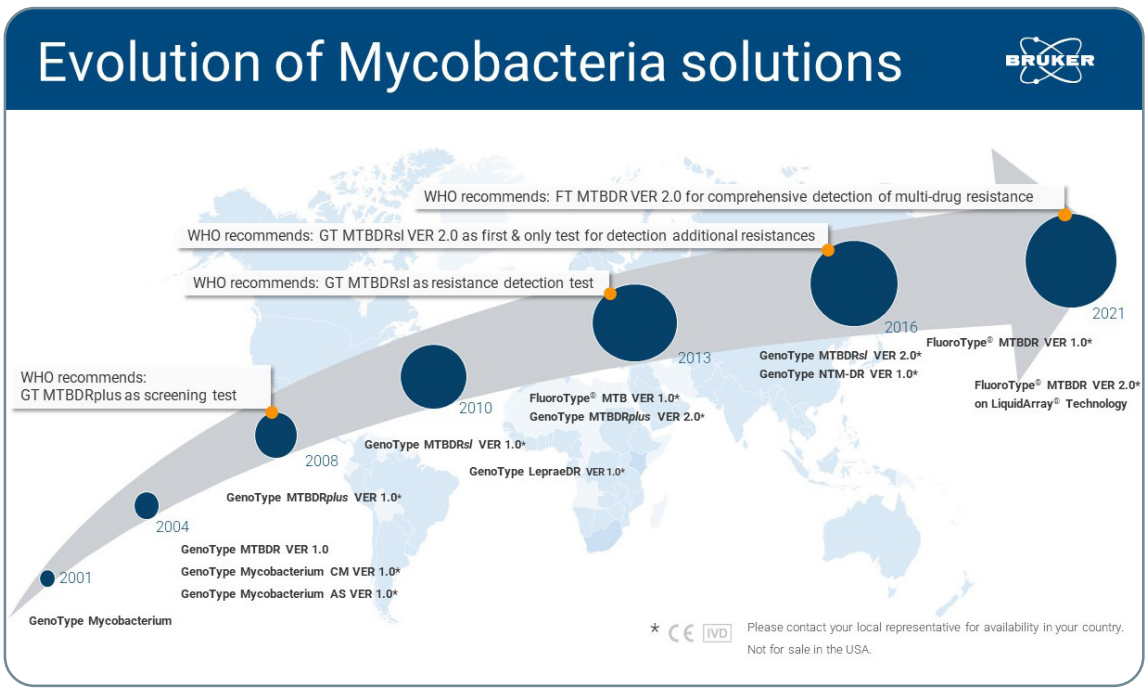
Prof. Warren has been a researcher at the SAMRC Centre at Stellenbosch University since 1996 and has brought the study of the molecular epidemiology of *M. tuberculosis* in South Africa to the forefront of international TB research. He became Unit Director of the SAMRC Centre for Tuberculosis Research in 2017, working in partnership with Stellenbosch University. There are 10 ongoing research thrusts at the Centre, with Prof. Warren leading the TB genomics thrust. He describes the role the team has played so far in TB research:

"We were right at the cutting edge of the molecular epidemiology of M. tuberculosis and, using the tools available, we attempted to uncover the driving force by charting the path of the epidemic we were observing in Cape Town. We found hundreds of different strains, many of which are clustered suggesting transmission was a key driver. We were one of the first research groups to show that a patient with a recurrent episode of TB is frequently re-infected with a different strain and that many TB cases harbor multiple different strains of TB in their lungs. Over time, our research has progressed towards studying drug resistant TB, which accounts for around 5% of the total epidemic. Our research shows that we need tools that will allow us to rapidly identify resistance with a minimum amount of subjectivity."

The shift to genetic testing

One of the biggest challenges in TB diagnostics is that *M. tuberculosis* grows very slowly, taking between one and two weeks to develop a culture and up to two months for susceptibility testing if the culture is positive. These conventional diagnostic testing methods require significant lab infrastructure and training. Therefore, research should evolve from classical microbiology, dependent on an organism's growth rate, to alternatives enabling the identification of the causative agent, as well as determining the presence of a genetic marker of susceptibility.

The GenoType MTBDR*plus* VER 2.0 line probe assay from Bruker Molecular Diagnostics – was one of the first genetic tests that moved away from conventional microbiology and phenotypic testing to confirm the presence of *M. tuberculosis* and of mutations conferring resistance to first-line drugs. Prof. Warren describes this as having caused a major mind shift amongst doctors who had relied on traditional methods to diagnose and treat TB for many years:



The portfolio of Bruker/Hain has a long history in mycobacteria research and development.

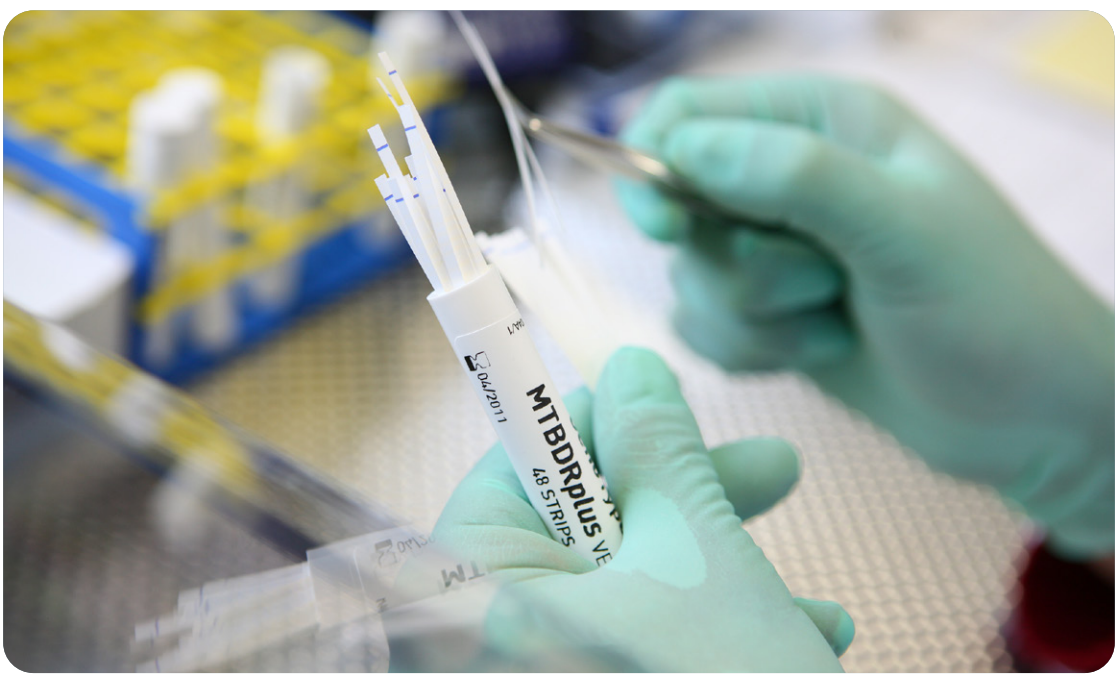
"Bruker's GenoType line probe assay was a key step in terms of re-engineering and improving clinical understanding of genetic markers of drug susceptibility. This, in a way, has been the starting point which has allowed us to investigate how much further we can go using genetic markers."

The GenoType MTBDR*plus* VER 2.0 assay accelerated time to diagnosis and treatment initiation and is a World Health Organization (WHO) endorsed commercial assay recommended for use on smear-positive specimens. It is easy to implement and doesn't require sophisticated, expensive instruments, which makes it a highly used assay in Africa.

How does the FluoroType® MTBDR VER 2.0 assay solve TB diagnosis challenges?

The FluoroType® MTBDR VER 2.0 was designed for high-throughput labs as a qualitative *in vitro* test for the semi-automated detection of *M. tuberculosis* and resistance to RIF and INH directly from sputum specimens or from cultivated samples. The assay is able to identify resistance through mutations of the *rpoB* gene and in the *katG* and *inhA/fabG1* promotor region. Prof. Warren and his colleagues at the SAMRC Centre evaluated the accuracy of the FluoroType® MTBDR VER 2.0 assay.[3] He describes his results:

"The FluoroType® showed extremely good performance in terms of detecting M. tuberculosis, detecting INH resistance and detecting RIF resistance. This assay performs extremely well both on samples with high bacillary load (smear-positive) and low bacillary load (smear-negative). It informed us in a single step which mutation was actually present – relevant as certain mutations confer different levels of resistance. For example, if there is an inhA promoter mutation, which confers low level INH resistance, the patient can possibly be treated with high levels of INH."



GenoType MTBDRplus VER 2.0 is based on PCR and the DNA•STRIP technology and allows the detection of M. tuberculosis complex and its resistance against rifampicin and isoniazid directly from clinical specimens.

The FluoroType® MTBDR VER 2.0 assay is a rapid molecular test that provides operator-independent results, helping to overcome the major hurdle of turnaround time and allowing the patient to start the correct treatment regimen as soon as possible. Prof. Warren describes the efficiency of the FluoroType® MTBDR VER 2.0 assay:

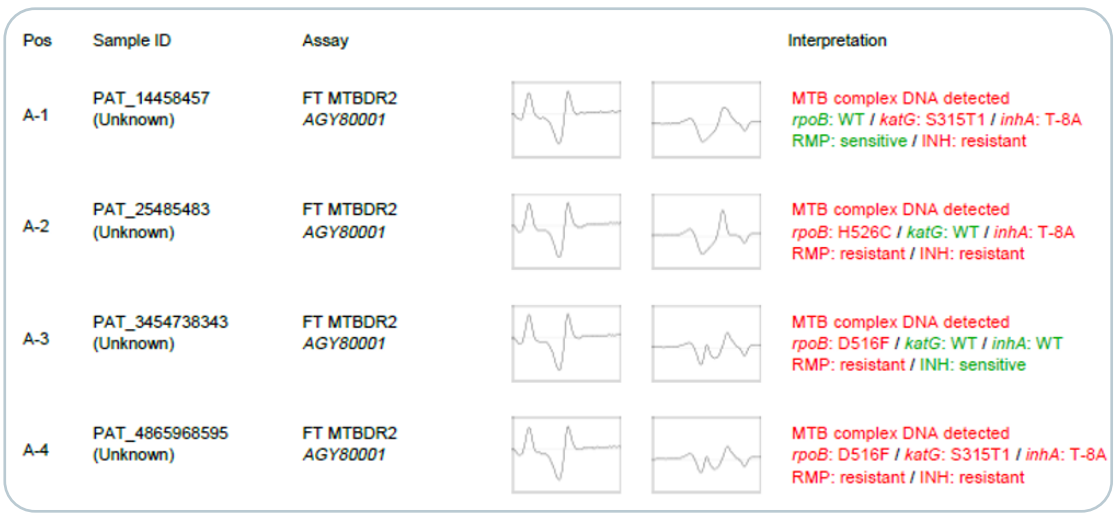
"The tests can all be done at the touch of a button. And it's rapid – this assay can give us an answer within three to four hours. The FluoroType® assay has been designed for the high volume, high throughput laboratory and is in the process of automating the whole procedure, from sputum specimen to performing amplification within the instrument. This is a dramatic time-saving on previous methods.

Using FluoroType® was a big step forward as the assay is contained within a single tube that is never opened, thereby allowing the assay to be run without specialist facilities.
I believe it is bridging a longstanding gap and helps us move towards a more precise way of diagnosing and treating TB."

The FluoroType® MTBDR VER 2.0 assay also removes the subjectivity of other methods, where visual inspection, either by the human eye or an instrument, is used to identify the presence or absence of a band. Not only did these manual reviewing steps slow the process down, but they also demanded more specialist knowledge. If there is an underlying population with a resistance marker that is not detected, then the patient may be treated before the underlying problem is identified, which could lead to treatment failure.

Prof. Warren discusses the benefits of Bruker introducing automated data interpretation:

"Bruker Molecular Diagnostics has removed the subjectivity of a visual inspection. Now, the algorithm reads this curve (or melt profile) and indicates a particular mutation. The machine learning algorithm produces a report, thereby removing both the possibility of subjectivity and the need for molecular genetics experts to operate the system."



Pos	Sample ID	Assay	Interpretation
A-1	PAT_14458457 (Unknown)	FT MTBDR2 *AGY80001*	MTB complex DNA detected *rpoB*: WT / *katG*: S315T1 / *inhA*: T-8A RMP: sensitive / INH: resistant
A-2	PAT_25485483 (Unknown)	FT MTBDR2 *AGY80001*	MTB complex DNA detected *rpoB*: H526C / *katG*: WT / *inhA*: T-8A RMP: resistant / INH: resistant
A-3	PAT_3454738343 (Unknown)	FT MTBDR2 *AGY80001*	MTB complex DNA detected *rpoB*: D516F / *katG*: WT / *inhA*: WT RMP: resistant / INH: sensitive
A-4	PAT_4865968595 (Unknown)	FT MTBDR2 *AGY80001*	MTB complex DNA detected *rpoB*: D516F / *katG*: S315T1 / *inhA*: T-8A RMP: resistant / INH: resistant

Result report of the FluoroType® MTBDR VER 2.0 assay result report

Collaborating with Bruker

Prof. Warren describes his partnership with Bruker as a two-way relationship:

"I think the world is desperate for new and more efficient diagnostics for both drug susceptible and DR-TB. While we are not a routine diagnostic lab, my team works together with Bruker Molecular Diagnostics to evaluate what we think are the most effective tools available.

Obviously, we'd like to see the assay evolve further, as programs across the world are developing new treatments for DR-TB. For example, we're now moving into the era of new drugs for treating RIF-resistant TB, so we need approved assays that will adapt."

Future developments in TB diagnosis

In 2020, the WHO recommended a new, shorter, and fully oral regimen for patients with MDR-TB.[2] The SAMRC team supports this move towards oral regimens for DR-TB, rather than injectable treatment, with South Africa leading the way. Moving away from injectable regimens would improve patient adherence. With changes in regimens comes the introduction of new drugs and MDR-TB testing methods will have to keep pace.

Recent work from SAMRC has demonstrated that as patients are prescribed newer drugs like bedaquiline, strains of TB can evolve different clones within the body. Prof Warren suggests that:

"TB is adapting to survive treatment; resistant sub-populations evolve out of a susceptible population and then replace the susceptible population leading to resistant TB. When both susceptible and resistant populations are present, this is termed hetero-resistance. The FluoroType® assay is able to detect the presence of underlying populations."

Bruker Development Outlook

Bruker's innovative LiquidArray® technology provides a powerful tool for the development of new FluoroType® assays, which could aid the detection of second-line antibiotics to treat MDR-TB and XDR-TB.

Further Reading

Click here for more information about the FluoroType® MTBDR VER 2.0 assay.

Click here for more information about the GenoType MTBDR*plus* VER 2.0 assay.

Click here for more information about the entire Mycobacteria assay portfolio.

References

1 World Health Organization: Global tuberculosis report 2021.
https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2021

2 World Health Organization: Tuberculosis Key Facts, 14 October 2020.
https://www.who.int/news-room/fact-sheets/detail/tuberculosis

3 Dippenaar, A., Derendinger, B., Dolby, T., Beylis, N., van Helden, P., Theron, G., Warren, R., de Vos, M.: "Diagnostic accuracy of the FluoroType MTB and MTBDR VER 2.0 assays for the centralized high-throughput detection of *Mycobacterium tuberculosis* complex DNA and isoniazid and rifampicin resistance," Clinical Microbiology and Infection, 1 September 2021.
https://doi.org/10.1016/j.cmi.2021.04.022

Expert Insights

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Hain Lifescience GmbH

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

info.mdx.de@bruker.com - www.hain-lifescience.de