Technical FAQs on Hain Genotype MTBDRplus Line Probe Assay

What is the Hain Genotype MTBDRplus and how does it work?

The Genotype MTBDRplus test is a WHO-endorsed line-probe assay (LPA) for the rapid detection of Mycobacterium tuberculosis complex (MTB) and mutations conferring resistance to rifampin (RIF) and isoniazid (INH) in AFB smear-positive sputum specimens. The purpose of LPA testing is to rapidly diagnose MDR-TB in known, smear-positive TB cases in which drug-resistance is suspected and on isolates of MTB grown in culture. Additionally, it can be used to detect mono-resistance with INH, and to confirm RIF resistance from other tests such as Xpert MTB/RIF.

The GenoType MTBDRplus test is based on DNA Strip technology. The test consists of three steps: DNA extraction from decontaminated sputum specimens/cultured material (solid/liquid medium); a multiplex PCR amplification; reverse hybridization where single-stranded amplicons bind to specific probes attached to the LPA strips. The visualised band patterns on the strips are then interpreted by either a manual comparison with a printed template or read and analysed in the GenoScan reader.

Please refer to the GenoType MTBDRplus kit insert for more details related to the procedure, precautions, interpretation, limitations and troubleshooting.

What is a line-probe assay and what other uses are there for LPAs?

LPAs are nitro-cellulose strips which have specific probes attached to them which are complementary to the DNA that is targeted in the specimen. There are other LPAs available for use on cultures that identify the various members of the MTB complex and up to thirty different non-tuberculous mycobacteria (NTM). Although currently not available via IPAQT, there is a LPA available for detection of resistance to second-line drugs (Genotype MTBDRsI).

Is the Genotype MTBDRplus test endorsed by the World Health Organization?

Yes, the WHO endorsed this technology for use on AFB smear-positive sputum specimens in 2008 and WHO policy is available at: [http://www.who.int/tb/features_archive/policy_statement.pdf](http://www.who.int/tb/features_archive/policy_statement.pdf)

How accurate is the Genotype MTBDRplus test?

A 2008 meta-analysis of publications by Ling D et al. (Eur Resp Journal 2008 [http://erj.ersjournals.com/content/32/5/1165.full.pdf](http://erj.ersjournals.com/content/32/5/1165.full.pdf)) showed that this test is highly accurate. When compared to culture and phenotypic DST the MTBDRplus has an approximate sensitivity and specificity for RIF resistance of 98.1% and 98.7% and for high-level INH resistance of 84.3% and 99.5%.

How is MTBDRplus different from conventional TB tests?
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Conventional tests for TB involve AFB smear, culture and DST. Microscopy cannot detect drug resistance, while the MTBDRplus can rapidly detect resistance to rifampicin and isoniazid, the most important first-line TB drugs. Culture and conventional DST take several weeks to produce results while the MTBDRplus can yield results on pulmonary samples for a batch of 12-48 specimens in approximately 6 hours. For smear-negative samples, once the specimen is grown in culture, the MTBDRplus can significantly shorten the time to resistance detection for rifampicin and isoniazid compared to conventional DST.

How is MTBDRplus different from the Xpert MTB/RIF assay?

The Xpert MTB/RIF assay (GeneXpert) is WHO approved for rapid detection of TB as well as rifampicin resistance. It can be directly performed on sputum samples of individuals with suspected TB, and can deliver results within 2 hours. There is no need for batching Xpert tests, since each cartridge can be run independently. Rifampicin resistance is used as a surrogate of MDR-TB.

The Genotype MTBDRplus assay is meant to be used on smear-positive pulmonary samples and on cultures from smear negative pulmonary TB cases. The goal is to rapidly diagnose MDR-TB. Unlike the Xpert test, the MTBDRplus test can detect resistance to both INH and RIF. There is no need for batching the Genotype test since each test is individually packaged as a single strip, but batching may be advantageous in reducing turnaround times.

On which samples can the Genotype MTBDRplus assay be run?

The assay is approved for use ONLY on AFB smear-positive sputum samples (spontaneous or induced sputum) or on culture isolates.

Can the Genotype MTBDRplus test be used on blood samples?

No, MTBDRplus CANNOT be used on blood samples. It is not validated for blood and will not produce interpretable results.

What about extrapulmonary (EPTB) samples?

The MTBDRplus has not been validated for use on EPTB samples but can be used to diagnose drug-resistance if MTB has already been isolated from EPTB samples.

How should the Genotype MTBDRplus results be interpreted?

<table>
<thead>
<tr>
<th>Result</th>
<th>Interpretation and suggested action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invalid result</td>
<td>This may be due to failure of the internal controls possibly due to inhibition of PCR or there may be an undecipherable band pattern due to improperly performed procedures. The assay will need to be repeated.</td>
</tr>
<tr>
<td>MTB not detected</td>
<td>No tuberculosis detected. However, since the sensitivity of MTBDRplus</td>
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</table>
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<table>
<thead>
<tr>
<th>MTB detected + RIF and INH resistance not detected*</th>
<th>This is diagnostic of drug-sensitive TB. Standard, short-course TB therapy can be initiated (6 months treatment), as per RNTCP/WHO guidelines.</th>
</tr>
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<tbody>
<tr>
<td>MTB detected + RIF and INH resistance detected*</td>
<td>This is highly suggestive of MDR-TB, but needs to be interpreted in the context of risk factors for drug-resistance. If the patient has been previously treated for TB or has failed first-line treatment, then risk of MDR-TB is high. In such a patient, a positive rifampicin (RIF) resistance result is sufficient to initiate second-line therapy for MDR-TB. At the same time, a sample should be sent for liquid culture and complete drug susceptibility testing (DST). When the DST results come back in about 2 – 3 weeks, the MDR-TB regimen can be modified/customized, based on DST profile.</td>
</tr>
<tr>
<td>MTB detected + INH (only) resistance detected*</td>
<td>This indicates possible mono-resistance to INH. While INH mono-resistance is not MDR-TB, such patients may have a higher risk of poor outcomes and amplification of drug resistance. Thus, a liquid culture \textit{plus} full DST should be ordered to customize treatment.</td>
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*It is important to notify all TB cases to the local governmental authorities, as per RNTCP guidelines.

It is important to note that line probe assays are not a complete replacement for conventional culture and DST. Mycobacteriological culture is still required for low bacterial load/smear-negative specimens while conventional DST is still necessary for full panel susceptibility testing and to detect XDR-TB.

**What are the limitations of the Genotype MTBDR\textit{plus} technology?**

According to a 2008 meta-analysis of published literature, the Hain Genotype MTBDR\textit{plus} has a sensitivity of 98.1% and 84.3% for RIF and INH respectively. This means it can miss TB cases which have very low levels of bacterial load. Thus, if the clinical picture is highly suggestive of TB, further testing is warranted.

An MTBDR\textit{plus} result that is positive for rifampicin and isoniazid resistance should be carefully interpreted and the risk of MDR-TB in a given patient and the expected prevalence of MDR-TB in a given setting should be considered. In patients with no risk factors for MDR-TB, a positive resistance result could indicate a false-positivity and must be interpreted cautiously. Likewise a test that shows no resistance to INH or RIF must be interpreted in the context of clinical findings such as treatment failure or risk factors for MDR-TB. Confirmatory DST testing is required in such cases.

The MTBDR\textit{plus} test requires a stable and uninterrupted electrical power supply for the PCR, hybridization and other instruments used. All instruments require annual calibration and maintenance. As with all open PCR tests, the laboratory must have separate work areas: a clean-room for reagent
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preparation and separate PCR and hybridization areas. Staff must be trained and adhere to PCR workflow protocols. Strict quality assurance is necessary to avoid amplicon contamination. A positive and negative external control should be run with each batch and there are 2 internal controls per strip.

Are there any dangers or precautions for staff?

According to WHO, processing of smear-positive specimens for direct testing with LPA should be performed in a BSL2 level laboratory, whereas performing the assay on positive cultures would require BSL3 facilities.

What measures are required for quality assurance and training?

LPAs must be performed only by accredited laboratories with expertise in molecular testing. Precautions to reduce the risk of cross-contamination of DNA molecular procedures are critical. This is achieved by using different rooms for DNA extraction, preparation of reagents for PCR (pre-amplification), PCR amplification and hybridization, and interpretation of results (post-amplification). Restricted access and uni-directional workflow through the rooms further reduce the likelihood of amplicon contamination. Careful cleaning of all rooms and equipment with 1% hypochlorite after each use is also critical.

Successful implementation and interpretation of line probe assays is highly dependent on the skill of laboratory staff performing the testing and the quality of supervision. Interpretation of results of line probe assays must be done with care due to the complexity of interpreting the banding patterns, however once the assay is established into routine use such interpretation becomes easier with a little experience. A high level of skill is required to interpret banding patterns in cases of unusual mutations or mixed mycobacterial populations. These issues must be covered in initial training, with access to ongoing access to technical support when unusual results are obtained. Post-training supervision and monitoring (ad hoc or remote) of staff by a senior person with expertise in molecular assays is therefore strongly recommended by the WHO.

Where do we find the product insert, operating procedures and training materials for running the assay?

http://www.ipaqt.org/testing-procedures/

How should providers and/or laboratories notify TB cases to the RNTCP/health authorities?

This is being worked out and IPAQT Secretariat will share the information to all labs in due course.

What is the external quality assurance (EQA) process for Genotype MTBDRplus?

While internal quality control should be executed continuously by laboratory staff, external quality assurance through blinded rechecking of subsets of specimens or proficiency testing by an independent external organization is strongly recommended by the WHO.
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The exact EQA process is being worked out and IPAQT Secretariat will share the information to all labs in due course.

Who should we contact for trouble-shooting, maintenance and other technical issues?

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