Truenat®

MTB-INH

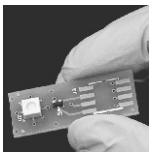
Chip-based Real Time PCR Test for Isoniazid Resistant
Mycobacterium tuberculosis

1. INTENDED USE

Truenat® MTB-INH (REF 601360005 / 601360020 / 601360025 / 601360050 / 601360100 / 601360200) is an automated point-of-care or near patient Chip-based Real Time Polymerase Chain Reaction (PCR) test for the detection of isoniazid resistance in *Mycobacterium tuberculosis* (MTB) in **Truenat® MTB/MTB Plus** positive human specimen and aids in the diagnosis of MDR-TB. This test detects the presence of major mutations (SNPs) in the MTB genome that are known to cause resistance to isoniazid. **Truenat® MTB-INH** runs on the **Truelab®** Real Time Quantitative micro PCR Analyzer. This is a follow on test, to be performed only on the extracted DNA from **Truenat® MTB/MTB Plus** positive sample. **Truenat® MTB-INH** is a single-use *in vitro* diagnostics test meant for professional use in near-patient, laboratory or any healthcare settings, by healthcare professionals or any user appropriately trained by a representative of Molbio Diagnostics.

2. INTRODUCTION

Multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB) has emerged as a major public health problem worldwide. The WHO estimates that in 2014 an estimated 4,80,000 people developed MDR-TB across the globe. MDR-TB is defined as TB that is resistant to both isoniazid (INH) and rifampicin (RMP). In many countries and regions, these resistant strains constitute a serious threat to the efficacy of tuberculosis control programs. One of the main reasons for treatment failure and fatal clinical outcome in patients with tuberculosis is resistance to rifampicin. Isoniazid resistance is most invariably associated with resistance to rifampicin. However many cases of MDR-TB because of isoniazid mono resistance are reported. Hence, detection of isoniazid resistance is recommended as a second most reliable surrogate marker for diagnosis of MDR-TB. Drug Susceptibility Testing (DST) by culture methods are the most common method used for detecting drug resistance. Culture methods require specialized and controlled laboratory facility and highly skilled manpower and takes 3 to 6 weeks to provide the result. Molecular techniques such as line probe assay and polymerase chain reaction (PCR) or Real Time PCR are accurate and much faster than culture. However, molecular techniques have so far been restricted to centralized reference laboratories, as they require skilled manpower and elaborate infrastructure. Also the turnaround time for results could take a few days.



The **Truenat®** point-of-care real time PCR system enables decentralization and near patient diagnosis of isoniazid resistant *Mycobacterium tuberculosis* by making real time PCR technology rapid, simple, robust and user friendly, offering "sample to result" capability even at resource limited settings. This is achieved through a combination of lightweight, portable, mains/battery operated **Truelab®** Real Time Quantitative micro PCR Analyzer and **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device and room temperature stable **Truenat®** micro PCR chip and **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit so that even the peripheral laboratories with minimal infrastructure and minimally trained technicians can easily perform these tests routinely in their facilities and report PCR results in less than an hour. Moreover, with these devices PCR testing can also be initiated in the field level, on site.

Truenat® MTB-INH is a disposable, room temperature stable, Chip-based Real Time PCR test with dried $MgCl_2$ in reaction well and freeze dried PCR reagents in microtube for performing Real Time PCR test for detection of isoniazid resistant *Mycobacterium tuberculosis* and runs on the **Truelab®** Real Time Quantitative micro PCR Analyzer. All components of **Truenat®** pouch are nuclease-free. It requires only six (6) µL of purified **Truenat® MTB/MTB Plus** positive DNA to be added to the reaction well for the analysis. The **Truenat® MTB-INH** chip also carries test and batch related information and stores information of used test to prevent any accidental re-use of the test.

NOTE: Truelab® / Truenat® / Trueprep® / Truepet® are all trademarks of Molbio Diagnostics Private Limited.

The Truelab® Real Time micro PCR Analyzer is protected by the following patents and patents granted: IN 2313/CHE/2007 (Patent No. 281573), WO2009/047804 and corresponding claims of any foreign counterpart(s) thereof.

The Truenat® micro PCR chip is protected by the following patents and patents pending: IN 2312/CHE/2007, WO 2009/047805 and corresponding claims of any foreign counterpart(s) thereof.

3. PRINCIPLE OF THE TEST

Truenat® MTB-INH works on the principle of Real Time Polymerase Chain Reaction. The extracted DNA from pre-treated patient sample assayed for MTB using **Truenat® MTB / Truenat® MTB Plus** test (refer **Truenat® MTB / Truenat® MTB Plus** pack insert) is run on the **Truelab®** Real Time Quantitative micro PCR Analyzer. If the sample tests positive for MTB, six (6) µL of the purified DNA is then dispensed using the provided calibrated micropipette and tip into the **Truenat® MTB-INH** microtube containing freeze dried PCR reagents and allowed to stand for 30-60 seconds to get a clear solution. ⚠ No mixing by tapping, shaking or by reverse pipetting should be done. Six (6) µL of this clear solution is then pipetted out using the same pipette and tip and dispensed into the reaction well of the **Truenat® MTB-INH** chip and the test is started. Isoniazid resistance detection is based on the principle of real time polymerase chain reaction followed by the probe melt analysis. Presence of mutations (SNPs) within the codon 315 of *katG* and promoter region of *inhA* is detected using a probe melt assay. A resistant sample causes a shift in the probe melt temperature (Tm). Assay includes a control probe. Tm of Control Probe is used for melt temperature correction. At the end of the test run, a INH Resistance detected or not detected result is displayed. The results can be printed via Bluetooth using the **Truelab®** micro PCR printer or transferred to the lab computer/or any remote computer via Wifi network or 4G/3G/GPRS network. Upto 20000 results can be stored in the **Truelab® Uno Dx/Duo/Quattro** analyzer for future recall and reference.

4. TARGET SELECTION

The target sequence for this assay is the *inhA* and *katG* genes of *Mycobacterium tuberculosis* genome.

5. CONTENTS OF THE Truenat® MTB-INH KIT

A. Individually sealed pouches
B. Package insert

Each individually sealed pouch contains:

1. **Truenat® MTB-INH** micro PCR chip (1 No.)
2. Microtube with freeze dried PCR reagents (1 No.)
3. DNase and RNase free pipette tip (1 No.)
4. Desiccant pouch (1 No.)

Pack sizes of **Truenat® MTB-INH KIT**

REF	601360005	601360020	601360025	601360050	601360100	601360200
Σ	5T	20T	25T	50T	100T	200T

6. STORAGE, HANDLING AND STABILITY

1. **Truenat® MTB-INH** test is stable for two (2) years from the date of manufacture if stored between 2-30°C. It is also stable for one (1) month at temperatures up to 45°C. Avoid exposure to light or elevated temperatures (above recommended levels). Do not freeze.
2. Do not open the pouch until ready to test. ⚠ Make sure to start the test promptly after 30-60 seconds of adding the elute to the microtube.
3. Do not use the pouch if torn.
4. Do not use pouches that have passed the expiration date.

7. MATERIALS REQUIRED BUT NOT PROVIDED WITH THE KIT

Truelab® Real Time micro PCR Workstation (REF623010001 / 633010001 / 643010001 / 653010001) consisting of,

1. **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device (REF603041001/603042001)
2. **Truelab® Uno Dx / Truelab® Duo / Truelab® Quattro** Real Time micro PCR Analyzer (REF 603021001/603022001/603023001).
3. **Truelab®** micro PCR Printer (REF 603050001).
4. **Truepet®** SPA fixed volume precision micropipette - 6 µl (REF 604070006).
5. **Truelab®** Microtube Stand (REF 603070001).

Also required additionally are: **Truenat® MTB/MTB Plus** Chip-based Real Time PCR test for *Mycobacterium tuberculosis*, **Trueprep® AUTO MTB** Sample Pre-treatment Pack (REF 60204AS05 / 60204AS20 / 60204AS25 / 60204AS50 / 60204AS100 / 60204AS200), **Trueprep® AUTO** Universal Cartridge Based Sample Prep Kit (REF 60203AR05 / 60203AR25 / 60203AR50 / 60203AR100 / 60203AR200) or **Trueprep® AUTO v2** Universal Cartridge Based Sample Prep Kit (REF 60207AR05 / 60207AR25 / 60207AR50 / 60207AR100 / 60207AR200), powder free disposable gloves, waste disposal container with lid and sodium hypochlorite.

8. SPECIMEN PREPARATION FOR EXTRACTION WITH Trueprep® AUTO/AUTO v2

Truenat® MTB-INH requires purified nucleic acids from sputum/EPTB specimen that are extracted using the **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device and **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit that have tested positive by **Truenat® MTB/MTB Plus** (Refer to the User Manual of **Trueprep® AUTO /**

AUTO v2 Universal Cartridge Based Sample Prep Device and the package inserts of **Trueprep® AUTO / AUTO v2** Universal Cartridge Based Sample Prep Kit, **Trueprep® AUTO MTB** Sample Pre-treatment Pack and **Truenat® MTB/MTB Plus** for details).

9. SAFETY PRECAUTIONS

1. For *in vitro* diagnostic use only.
2. Bring all reagents and specimen to room temperature (20-30°C) before use.
3. Do not use kit beyond expiry date.
4. Carefully read the user manuals, package inserts and Material Safety Data Sheets (MSDS) of all the components of the **Truenat®** point-of-care real time PCR system before use.
5. Good laboratory practices are recommended to avoid contamination of specimens or reagents.
6. All materials of human origin should be handled as though potentially infectious.
7. Do not pipette any material by mouth.
8. Do not eat, drink, smoke, apply cosmetics or handle contact lenses in the area where testing is done.
9. Use protective clothing and wear disposable gloves when handling samples and while performing sample extraction.
10. Do not substitute assay components / reagents with any other components / reagents.
11. Each single-use **Truenat®** chip is used to process one test. Do not reuse chip.

10. PROCEDURAL PRECAUTIONS

1. Check all packages before using the kit. Damage to the packaging does not prevent the contents of the kit from being used. However, if the outer packaging is damaged the user must confirm that individual components of the kit are intact before using them.
2. Do not perform the test in the presence of reactive vapours (e.g. from Sodium hypochlorite, acids, alkalis or aldehydes) or dust.
3. While retrieving the **Truenat® MTB-INH** micro PCR chip, microtube and the DNase and RNase free pipette tip from the pouch, ensure that neither bare hands nor gloves that have been used for previous tests run are used.
4. Ensure that the colour of the desiccant pouch is orange after opening a sealed **Truenat®** chip pouch. If the colour of the desiccant pouch changes from orange to white due to the absorption of moisture, do not use the contents of the **Truenat®** chip pouch.

11. PROCEDURAL LIMITATIONS

1. Optimal performance of this test requires appropriate specimen collection, handling, storage and transport to the test site.
2. Though very rare, mutations within the highly conserved regions of the target genome where the **Truenat®** assay primers and/or probe bind may result in the under-quantitation of or a failure to detect the presence of the concerned pathogen.
3. The instruments and assay procedures are designed to minimize the risk of contamination by PCR amplification products. However, it is essential to follow good laboratory practices and ensure careful adherence to the procedures specified in this package insert for avoiding nucleic acid contamination from previous amplifications, positive controls or specimens.
4. A specimen for which the **Truenat®** assay reports "Not Detected" cannot be concluded to be negative for the concerned pathogen. As with any diagnostic test, results from the **Truenat®** assay should be interpreted in the context of other clinical and laboratory findings.

12. CLEANING AND DECONTAMINATION

1. Spills of potentially infectious material should be cleaned up immediately with absorbent paper tissue and the contaminated area should be decontaminated with disinfectants such as 0.5% freshly prepared Sodium hypochlorite [10 times dilution of 5% Sodium hypochlorite (household bleach)] before continuing work.
2. Sodium hypochlorite should not be used on an acid-containing spill unless the spill-area is wiped dry first. Materials used to clean spills, including gloves should be disposed off as potentially bio-hazardous waste e.g. in a bio-hazard waste container.

13. TEST PROCEDURE

The **Truenat® MTB-INH** test is a follow-on test to be performed using the nucleic acid elute extracted from the **Truenat® MTB/MTB Plus** positive samples. (Please also refer the **Truelab®** Real Time Quantitative micro PCR Analyzer user manual)

1. Switch on the **Truelab®** analyzer.
2. Select Username and enter password.
3. For **Truelab® Uno Dx**, select the test profile for "MTB-INH" to be run from the profiles screen on the analyzer screen. For **Truelab® Duo/Quattro**, select the bay (I/II) for **Duo** and (I/II/III/IV) for **Quattro** from the status screen to view the profiles screen. Select the test profile for "MTB-INH" to be run from the profiles screen, on the analyzer screen.
4. Enter the patient details as prompted in the **Truelab®** analyzer screen.
5. Press start reaction.
6. For **Truelab® Uno Dx,** press the eject button to open the chip tray. For **Truelab® Duo/Quattro**, the chip tray opens automatically on tapping the "Start Test" button.
7. Open a pouch of **Truenat® MTB-INH** and retrieve the micro PCR chip, microtube and DNase and RNase free pipette tip. Do not open the pouch until ready to test.
8. Place the **Truenat® MTB-INH** chip on the chip tray without touching the white reaction well. The reaction well should be facing up and away from the analyzer. Gently place the chip on the chip tray by aligning it in the slot provided.
9. Place the microtube containing freeze dried PCR reagents in the microtube stand provided along with the **Truelab®** Real Time micro PCR workstation after ensuring that white pellet of freeze dried PCR reagents remains at the bottom of the microtube. Remove the microtube cap and dispose it off as per the section on "Disposal and Destruction" (Section 16). Using the filter barrier tip provided in the pouch, pipette out six (6) µL of the purified DNA from the elute collection tube into the microtube. Allow it to stand for 30-60 seconds (in-use time) to get a clear solution. ⚠ Do not mix it by tapping, shaking or by reverse pipetting. Using the same filter barrier tip, pipette out six (6) µL of this clear solution and dispense into the centre of the white reaction well of the **Truenat® MTB-INH** chip. Take care not to scratch the internal well surface and not to spill elute on the outside of the well. Dispose off the microtip as per the section on "Disposal and Destruction"(Section 16).
10. For **Truelab® Uno Dx**, slide the chip tray containing the **Truenat® MTB-INH** Chip-based Real Time PCR test loaded with the sample into the **Truelab®** analyzer. Press "YES" on the "Please Load Sample" prompt. For **Truelab® Duo/Quattro**, select "YES" at the "Please Load Sample" prompt. Chip tray will close automatically and the reaction will start. ⚠ Make sure to start the test promptly after 30-60 seconds of adding the elute to the microtube.
11. Read the result from the screen.
12. After the reaction is completed, for **Truelab® Uno Dx,** push the eject button to eject the chip tray. For **Truelab® Duo/Quattro**, tap the "Open/Close Tray" button to eject the chip tray.
13. Take out the **Truenat® MTB-INH** micro PCR chip at end of the test and dispose it off as per the section on "Disposal and Destruction" (Section 16).
14. Turn on **Truelab®** micro PCR printer and select print on the screen for printing out hard copy of the results. Test results are automatically stored and can be retrieved any time later. (Refer to **Truelab®** analyzer manual).
15. Switch off the **Truelab®** analyzer.

14. RESULTS AND INTERPRETATIONS

This test is based on real time PCR coupled with Probe Melt analysis. The results and interpretations are based on the melting temperature (Tm) values of the probes complementary to *inhA* and *katG* genes. At the end of the test run, the results screen will display "High INH resistance detected" if mutations are detected in *katG* only or in both *inhA* and *katG*; "Low INH resistance detected" if mutations are detected in *inhA* only; "INH Resistance not detected" if mutations are not detected. Indeterminate will be displayed when the obtained Tm values don't meet the requirements for resistance calculation, an indeterminate test has to be repeated using same MTB/MTB plus elute.

15. QUALITY CONTROL PROCEDURES

To ensure that the **Truelab®** Real Time micro PCR Analyzer is working accurately, run known PCR positive and negative samples from time to time.

16. DISPOSAL AND DESTRUCTION

1. Submerge the used **Truenat® MTB-INH** chip, microtube, microtube cap, pipette tips etc. in freshly prepared 0.5% sodium hypochlorite solution for 30 minutes before disposal as per the standard medical waste disposal guidelines.
2. Disinfect the solutions and/or solid waste containing biological samples before discarding them according to local regulations.
3. Samples and reagents of human and animal origin, as well as contaminated materials, disposables, neutralized acids and other waste materials must be discarded according to local regulations after decontamination by immersion in a freshly prepared 0.5% of sodium hypochlorite for 30 minutes (1 volume of 5% Sodium hypochlorite for 10 volumes of water).
4. Do not autoclave materials or solutions containing Sodium hypochlorite.
5. Chemicals should be handled in accordance with Good Laboratory Practice and disposed off according to the local regulations.

17. SPECIFIC PERFORMANCE CHARACTERISTICS

Analytical Exclusivity (Primer Specificity):

Genomic DNA sequence of following microorganisms were evaluated *in silico* from the NCBI database using the NCBI nucleotide blast and primer blast tools to determine for potential cross-reactivity in the **Truenat® MTB-INH** assay. *In-silico* analysis showed no potential amplicons using the oligos used in **Truenat® MTB-INH** test with the listed organisms.

Organisms	***Organisms***	***Organisms***
Cytomegalovirus	*Mycobacterium gordonae*	*Trichomonas vaginalis*
Adenovirus	*Mycobacterium avium*	*Escherichia coli*
Hepatitis B Virus	*Mycobacterium fortuitum*	*Streptococcus mutans*
Hepatitis C Virus	*Mycobacteroides abscessus*	*Staphylococcus aureus*
Human Immunodeficiency virus	*Mycobacterium ulcerans*	*Enterobacter cloacae*
Epstein–Barr virus	*Mycobacterium scrofulaceum*	*Chlamydia trachomatis*
Herpes Simplex Virus	*Mycobacterium intracellulare*	*Candida albicans*
Simian virus	*Mycobacterium malmoense*	*Acinetobacter anitratus*
Mycobacterium kansasii	*Neisseria gonorrhoeae*	
Mycobacterium szulgai	*Enterococcus faecalis*	

Analytical specificity/Cross reactivity:

The potential for false positive results arising from cross-reactivity with other organisms unrelated to *Mycobacterium tuberculosis* was determined by spiking negative sputum elute with concentration of 10^6 CFU/mL of non-mycobacterial DNA. The results showed no cross-reactivity of **Truenat® MTB-INH** to any of the non-mycobacterial organisms tested.

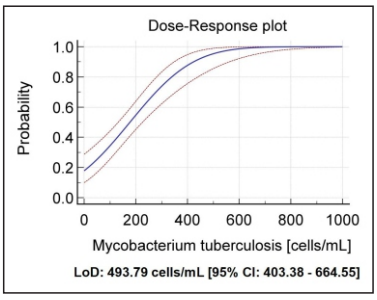
Linearity:

The linearity analysis was performed according to CLSI Guidelines. Serial dilutions of H37Rv, ZeptoMetrix stock from 5E+06 to 5E+02 cells/mL were made and tested using **Truenat® MTB-INH** on **Truelab®** Real Time micro PCR Analyzer. The assay is found to be linear over 5 orders of magnitude. (from 5E+06 to 5E+02 cells/mL) for *Mycobacterium tuberculosis* quantified H37Rv from Zeptometrix by **Truenat® MTB-INH** test.

Limit of detection (LoD):

MTB strain H37Rv from Zeptometrix was used for LoD determination. The LoD was determined by testing **Trueprep® AUTO** Universal Cartridge Based Sample Prep Device extracts, using dilutions of H37Rv strain (1000 cells/mL, 500 cells/mL, 300 cells/mL, 200 cells/mL, 100 cells/mL, 50 cells/mL, 25 cells/mL, 0 cells/mL) in negative sputum. Each dilution was tested as indicated in following table. Probit analysis of the data was used to determine the concentration with 95% probability of detection. The LoD was found to be 494 cells/mL of sputum for **Truenat® MTB-INH** assay.

Cells/mL	Total runs	Detected
1000	24	24
500	24	21
300	24	20
200	24	16
100	24	9
50	24	6
25	24	2



Robustness:

Potential sample carryover within the **Truenat® MTB-INH** test was evaluated by testing alternate positive followed by negative samples. 10 positive samples and 10 negative samples were used for the study. The **Truenat® MTB-INH** test did not exhibit detectable carryover contamination from positive to negative samples.

Reproducibility:

The purpose of this study is to compare the functional performance of the **Truenat® MTB-INH** assay using three different titres of samples on **Truelab® Quattro** Real Time micro PCR analyzer. The samples were extracted on **Trueprep® AUTO** Universal Cartridge Based Sample Prep Device and tested among three different users (Inter user), on three different devices (Inter device) and on 5 consecutive days (Inter day) to check the variability. Mean %CV values for all titres has been calculated for *inhA* as Inter User (0.36), Inter day (0.41) and Inter Device (0.26) while for *KatG* as Inter User (0.20), Inter day (0.37) and Inter Device (0.25) which were in the accepted range of ≤15% CV for **Truenat® MTB-INH** assay.

Accuracy

Accuracy was determined by performing DNA extractions and PCR for characterized samples from TDR Bank, ITM, Antwerp in **Truenat® MTB-INH.** The results of **Truenat® MTB-INH** assay was in concordance with the characterized panel data.

Precision

Precision was tested by performing **Truenat® MTB-INH** assay with extracted DNA of High (2.0E+06 CFU/ml), Medium of (1.5E+05 CFU/ml) and Low (9.0E+03 CFU/ml) DNA for five consecutive days. Every day PCR for each titre DNA was run in triplicates. The %CV values obtained for *inhA* as High titre (0.35), Medium titre (0.24) and Low titre (0.30) while for *KatG* as High titre (0.34), Medium titre (0.25) and Low titre (0.31) were within the accepted range of ≤15% CV for **Truenat® MTB-INH** assay.

Clinical Validation:

A panel of 50 M.tb isolates comprising of 25 known Isoniazid resistant and 25 known Isoniazid sensitive were run in parallel on three different lots of **Truenat® MTB-INH** assay at National Institute for Research in Tuberculosis, Chennai against the GenoType MTBDRplus line probe assay. Discrepancy resolution of the one "false positive" sample by genome sequencing showed this to be a Hetero infection that was not picked up by the comparator assay. No variation was observed across three lots of **Truenat® MTB-INH** chips tested. The **Truenat® MTB-INH** test was found to have a sensitivity of 100% and a specificity of 96.15%.

18. REFERENCES

1. World Health Organization. (N.d.). Global Tuberculosis Report. Retrieved from https://www.who.int/tb/publications/global_report/en/
2. Stagg, H.R., Lipman, M.C., McHugh, T.D., et al. (2017). Isoniazid resistant tuberculosis - a cause for concern?. International Journal of Tuberculosis and Lung Disease, 21(2), 129–139.
3. Jhun, W.B., and Koh, J.W. (2020). Treatment of Isoniazid-Resistant Pulmonary Tuberculosis. Tuberculosis and Respiratory Diseases, 83(1), 20-30.
4. Kim, S.J. (2005). Drug-susceptibility testing in tuberculosis: methods and reliability of results. European Respiratory Journal, 25, 564-569.
5. Ahmad, S., and Mokaddas, E. (2009). Recent advances in the diagnosis and treatment of multidrug-resistant tuberculosis. Respiratory Medicine, 103, 1777-1790.

SYMBOL KEYS

 Consult instruction for use.	 *In vitro* Diagnostic Medical Device. Not for medicinal use.	 Batch number/ Lot number.	 Catalogue number.	 Unique Device Identifier.	 This way up.	 Manufacturer.	 Caution.	 Non sterile.
 Contains sufficient for <n> tests	 Temperature limitations.	 Date of manufacture.	 Date of expiry.	 For single use only.	 Keep dry.	 Keep away from sunlight	 Device for near-patient testing	

Molbio Diagnostics Private Limited

Registered Office & Manufacturing Unit:

Plot No. L-46, Phase II D,
Verna Industrial Estate, Verna,
Goa - 403 722, INDIA

www.molbiodiagnostics.com

Email: sales@molbiodiagnostics.com (Sales Enquiries)
customersupport@molbiodiagnostics.com (Feedback and Customer Support)

TNINH/0324/V-04
English - March 2024