CE **Truenat®**

MTB

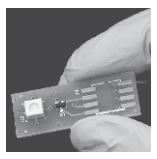
Chip-based Real Time PCR Test for *Mycobacterium tuberculosis*

1. INTENDED USE

Truenat® MTB (REF 601030005 / 601030020 / 601030025 / 601030050 / 601030100 / 601030200) is an automated point-of-care or near patient Chip-based Real Time Polymerase Chain Reaction (PCR) test for the quantitative detection and diagnosis of *Mycobacterium tuberculosis* (MTB) in human pulmonary and EPTB specimen and aids in the diagnosis of infection with MTB. **Truenat® MTB** runs on the **Truelab®** Real Time Quantitative micro PCR Analyzers. **Truenat® MTB** 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

Tuberculosis (TB) is an infectious disease caused predominantly by the bacillus *Mycobacterium tuberculosis.* It typically affects the lungs (pulmonary TB) but can affect other sites as well (extra pulmonary TB). Tuberculosis (TB) is the second largest killer worldwide, after HIV and is the leading cause of death in HIV patients. Pulmonary TB spreads through air and is highly contagious. Over 80% of TB infections are pulmonary and if left untreated, a pulmonary TB patient can infect up to 10-15 other people through close contact over the course of a year. Due to the highly infectious nature of pulmonary TB, it is important to diagnose and treat the disease very early. Despite the availability of highly effective treatment for decades, TB remains a major global health problem mainly because of poor case detection. The most common method for diagnosing pulmonary TB worldwide is sputum smear microscopy. However sensitivity of direct smear microscopy is low and estimates range from 30% to 70%. It is even lower in case of HIV-infected patients. Culture is more sensitive than microscopy and is considered the current gold standard. Culture requires specialized and controlled laboratory facility and highly skilled manpower and takes 3 to 6 weeks to provide the result. Molecular techniques such as polymerase chain reaction (PCR) or Real Time PCR are much more sensitive than microscopy and culture. However PCR or Real Time PCR tests 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 **Truelab®** Real Time Quantitative micro PCR System enables decentralization and near patient diagnosis of MTB by making real time PCR technology rapid, simple, robust and user friendly and offering "sample to result" capability even at resource limited settings. This is achieved through a combination of light weight, 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 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 *Mycobacterium tuberculosis* and runs on the **Truelab®** Real Time Quantitative micro PCR Analyzer. It requires only six (6) µL of purified DNA to be added to the reaction well for the analysis.

The intelligent chip also carries test and batch related information including standard values for quantitation. The **Truenat® MTB** Chip-based Real Time PCR test also 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. The Truenat® MTB Chip-based Real Time PCR test is protected by the following patents and patents pending: IN 796/CHE/2012 and corresponding claims of any foreign counterpart(s) thereof.

3. PRINCIPLE OF THE TEST

Truenat® MTB works on the principle of Real Time Polymerase Chain Reaction based on Taqman chemistry. The DNA from the patient sample is first extracted using **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device and **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit. The **Truenat® MTB** chip is placed on the chip tray of the **Truelab®** Real Time Quantitative micro PCR Analyzer. Six (6) µL of the purified DNA is then dispensed using the provided micropipette and tip into the 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** chip and the test is started. A positive amplification causes the dual labeled fluorescent probe in the **Truenat® MTB** Chip-based Real Time PCR test to release the fluorophores in an exponential manner which is then captured by the built-in opto-electronic sensor and displayed as amplification curve on the analyzer screen, on a real time basis during the test run. The Cycle threshold (Ct) is defined as the number of amplification cycles required for the fluorescent signal to cross the threshold (i.e. exceed the background signal). Ct levels are inversely proportional to the amount of target nucleic acid in the sample. (i.e. the lower the Ct level the greater is the amount of target nucleic acid in the sample). In the case of negative samples, amplification does not occur and a horizontal amplification curve is displayed on the screen during the test run. At the end of the test run, a MTB "DETECTED" or "NOT DETECTED" result is displayed and in positive cases, quantitative values is also displayed on the screen. Based on the Ct of the internal positive control (IPC), the validity of the test run is also displayed. The IPC is a full process control that undergoes all the processes the specimen undergoes - from extraction to amplification thereby validating the test run from sample to result. Absence of or shift of IPC Ct beyond a pre-set range in case of negative samples invalidates the test run. While IPC will co-amplify in most positive cases also, in some specimen having a high target load, the IPC may not amplify, however the test run is still considered valid. 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 3G/GPRS network. Upto 20,000 results in **Truelab® Uno Dx/ Duo/Quattro** can be stored on the analyzer for future recall and reference.

4. TARGET SELECTION

The target sequence for **Truenat® MTB** is *nrd*B gene which codes for ribonucleoside- diphosphate reductase large subunit.

5. CONTENTS OF THE Truenat® MTB KIT

A. Individually sealed pouches

B. Package Insert

Each individually sealed pouch contains:

1. **Truenat® MTB** micro PCR chip (1 Nos.)
2. Microtube with freeze dried PCR reagents (1 Nos.)
3. DNase & RNase free pipette tip (1 Nos.)
4. Desiccant pouch (1 Nos.)

REF	601030005	601030020	601030025	601030050	601030100	601030200
Σ	5T	20T	25T	50T	100T	200T

6. CONTENTS OF Trueprep® AUTO MTB Sample Pre-treatment Pack

A. Liquefaction buffer

B. Lysis buffer

C. Disposable transfer pipette (graduated)

D. Package Insert.

REF	60204AS05	60204AS20	60204AS25	60204AS50	60204AS100	60204AS200
Σ	5T	20T	25T	50T	100T	200T

7. STORAGE AND STABILITY

Truenat® MTB chip 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.

Trueprep® AUTO MTB Sample Pre-treatment Pack is stable for two (2) years from the date of manufacture if stored between 2-40°C. It is also stable for one (1) month at temperatures up to 45°C. Do not freeze.

8. MATERIALS REQUIRED BUT NOT PROVIDED WITH THE KIT

Truelab® Real Time micro PCR Workstation (REF 623010001 / 633010001 / 643010001 / 653010001) consisting of

1. **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device (REF 603041001 / 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: **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), **Truenat®** Positive Control Kit - Panel I (REF 801010008), powder free disposable gloves, waste disposal container with lid.

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

Truenat® MTB requires purified nucleic acids from pulmonary and 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. Samples must be liquefied and pre-treated using the **Trueprep® AUTO** MTB Sample Pre-treatment Pack provided (Refer to the package insert of **Trueprep® AUTO MTB Sample Pre-treatment Pack** for details) before proceeding for extraction.

Sample Storage and Transportation:

Sample pre-treatment decontaminates the specimen and makes it ready for extraction. Sample in this form is stable for 3 days at upto 40°C and 1 week at 30°C .

Nucleic acid extraction: Follow extraction procedure (Section-13) of **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit package insert. (Refer to the User Manual of **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device and the package insert of **Trueprep® AUTO** Universal Cartridge Based Sample Prep Kit for details). ⚠ Dispose off lysis buffer tube and transfer pipette after use, as per the section on "Disposal and Destruction" (Section 17).

10. 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 **Truelab® Real Time micro PCR System** before use
5. All materials of human origin should be handled as potential infection hazards.
6. Do not pipette any material by mouth.
7. Do not eat, drink, smoke, apply cosmetics or handle contact lenses in the area where testing is done.
8. Use protective clothing and wear disposable gloves when handling samples and while performing sample extraction.

11. 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** micro PCR chip, microtube and the DNase & 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 dessicant 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.

12. 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.

13. 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 biohazard waste container.

14. TEST PROCEDURE

(Please also refer the **Truelab®** Real Time 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" 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" 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 Test.
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** and retrieve the micro PCR chip, microtube and DNase & RNase free pipette tip. Do not open the pouch until ready to test.
8. Place the **Truenat® MTB** 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 17). 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** 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 17).
10. For **Truelab® Uno Dx**, slide the chip tray containing the **Truenat® MTB** 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** micro PCR chip at end of the test and dispose it off as per the section on "Disposal and Destruction" (Section 17).
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.

15. RESULTS & INTERPRETATIONS

Two amplification curves are displayed on the **Truelab®** analyzer screen when optical plot is selected to indicate the progress of the test. Both the target and the internal positive control (IPC)* curves will take a steep, exponential path when the fluorescence crosses the threshold value in case of positive samples. The Cycle threshold (Ct) will depend on the number of target nucleic acids in the sample. The target curve will remain horizontal throughout the test duration and the IPC curve will take an exponential path in case of negative samples. In case the IPC curve remains horizontal in a negative sample, the test is considered as invalid. At the end of the test run, the results screen will display "DETECTED" for Positive result** or "NOT DETECTED" for Negative result. The result screen would also display the Ct value and the colony forming units per milliliter (CFU/ml) for positive specimen. The result screen also displays the validity of the test run as "VALID" or "INVALID". Invalid samples have to be repeated with fresh specimen from the sample preparation stage.

*Note: IPC will co-amplify in most positive cases also, in some specimen having a high target load, the IPC may not amplify, however the test run is still considered valid.

In case of **Truenat® MTB DETECTED (Positive) result, proceed to run the follow-on **Truenat® MTB-RIF Dx** test, using the 'MTB-RIF' tab on the result page, for detection of Rifampicin resistance in *Mycobacterium tuberculosis*.

16. QUALITY CONTROL PROCEDURES

To ensure that the **Truelab®** analyzer is working accurately, run positive and negative controls from time to time. The **Truenat®** Positive Control Kit Panel - I (REF 801010008) containing Positive Control and Negative Control must be ordered separately. It is advisable to run controls under the following circumstances:

● Whenever a new shipment of test kits is received. ● When opening a new test kit lot. ● If the temperature of the storage area falls outside of 2-30° C. ● By each new user prior to performing testing on clinical specimen.

17. DISPOSAL AND DESTRUCTION

1. Submerge the used **Truenat® MTB** chip, microtube, microtube cap, transfer pipette, 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.

18. SPECIFIC PERFORMANCE CHARACTERISTICS

Analytical Exclusivity (Primer Specificity): Genomic DNA sequence of following microorganisms were evaluated *in silico* from the the NCBI database using the NCBI nucleotide blast and primer blast tools to determine for potential cross-reactivity in the **Truenat® MTB** assay. No cross reactivity in the performance of the **Truenat® MTB** assay was observed with the below listed microorganisms.

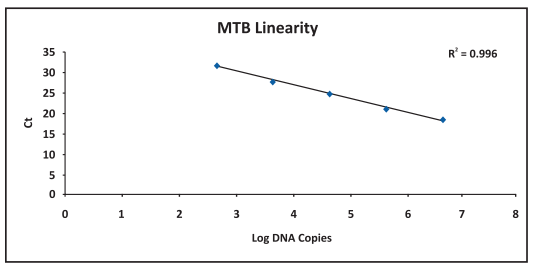
Acinetobacter anitratus	*Gardnerella vaginalis*	*M. abscessus*	Hepatitis B virus
Candida albicans	*Trichomonas vaginalis*	*M. fortuitum*	Hepatitis C virus
Chlamydia trachomatis	*Enterococcus faecalis*	*M. avium*	Human Immunodeficiency virus
Enterobacter cloacae	*Neisseria gonorrhoeae*	*M. gordonae*	Epstein–Barr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

In-silico analysis showed no potential amplicons using the oligos used in **Truenat® MTB** test with the listed organisms.

Analytical Inclusivity: To verify detection of strains in TDR-TB-Strain Bank in **Truenat® MTB** assay 235 strains in the TDR-TB-Strain Bank were tested in a blind study to check the inclusivity of **Truenat® MTB** against a panel of diverse MTB strains. **Truenat® MTB** identified all 233 *M. tuberculosis* strains and obtained a negative results for the 2 non-tuberculosis mycobacterial strain that were included (*M. terrae* and *M. avium*) in the test panel. **Truenat® MTB** correctly identified MTB and non-MTB strains in the TDR-TB-strain Bank panel.

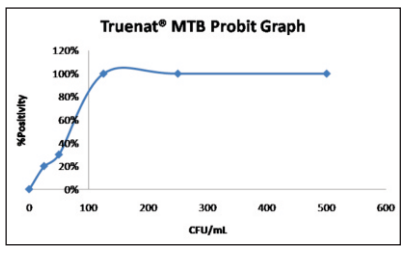
Analytical Specificity (Interference study) : For this study low load sample has been used. To the sample, blood was spiked to 10-30% and then the sample was subjected to sample prep on **Trueprep® AUTO**. DNA was eluted and PCR was performed on **Truelab® Uno Dx** using **Truenat® MTB** assay. The presence of blood till 30% did not interfere with the performance of **Truenat® MTB** assay. assay.

Linearity: Serial dilutions of Zepto *M. Tuberculosis* H37Rv were made from 5.0E+06 to 5.0E+02 and nucleic acids were extracted on **Trueprep® AUTO** Universal Cartridge Based Sample Prep Device followed by PCR on **Truelab®** Real Time micro PCR Analyzer. The **Truenat® MTB** test is found to be linear over 5 orders of magnitude (from 5.0E+06 to 5.0E+02 CFU/mL) for Zepto *M. Tuberculosis* H37Rv.



Limit of Detection (LoD): The LoD was determined by making ZeptoMetrix H37Rv sample and performing nucleic acid extractions on **Trueprep® AUTO** Universal Cartridge Based Sample Prep Device for each of the dilution 10 times followed by PCR on **Truelab® Uno Dx**. Probit analysis of the data was used to determine the concentration of the DNA with 95% probability of detection. The LoD was found to be 100 CFU/mL for ZeptoMetrix H37Rv sample.

CFU/ml	% Positivity
500	100%
250	100%
125	100%
50	30%
25	20%
0	0%



Robustness: To determine whether the **Truenat® MTB** Chip-based Real Time PCR test showed any signs of carryover between the runs, alternate positive and negatives sputum samples were extracted and further tested the same by PCR. 20 positive samples and 20 negative samples were used for the study. The **Truenat® MTB** test did not exhibit detectable carryover from positive to negative samples.

Reproducibility: The purpose of this study is to compare the functional performance of the **Truenat®** MTB assay using three different titres of samples on **Truelab® Uno Dx** Real Time Quantitative micro PCR Analyzer. High, Medium and low titre 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 Inter User (1.53), Inter day (1.74) and Inter Device (1.02) which were in the accepted range of ≤15% CV for **Truenat® MTB** assay.

Effect of potentially interfering substances: A study was conducted to determine the effect of potentially interfering substances on the performance of **Truenat® MTB** test. This was determined by spiking potentially interfering substances such as Kanamycin (5 mg/mL), Amoxicillin (5 mg/mL), Levofloxacin (5 mg/mL), Ethambutol (60 µg/mL), Pyrazinamide (500 µg/mL), Dexamethasone (5 µg/mL), Zanamivir (50 µg/mL), Mupirocin (5%), Rifampicin (120 µg/mL), Budesonide (5 µg/mL), Beclomethasone (5 µg/mL), Triamcinolone (5 µg/mL) Mometasone (5 µg/mL), Fluticasone (5 µg/mL), Mucin (5%), Blood (5%), DNA (10^6 cells/mL), Isoniazid (90 mg/mL), Streptomycin (400 µg/mL), Epinephrine (1 mg/mL), into negative sputum as well as positive (250 copies/mL of MTB) sputum samples. Both the MTB negative and positive samples were subjected to sample preparation on **Trueprep® AUTO** Universal Cartridge Based Sample Prep Device followed by PCR on **Truenat® MTB** test in triplicates runs. The performance of the **Truenat® MTB** assay was not affected by the potentially interfering substances mentioned under the stated experimental conditions.

Precision: Precision was tested by performing **Truenat® MTB** assay with extracted DNA from sputum of High (2.00E+06 copies/mL), Medium (2.00E+04 copies/mL) and Low (2.00E+02 copies/mL) for five consecutive days. Every day PCR for each titre DNA was run in duplicates. The %CV values obtained for High titre (2.9), Medium titre (1.55) and low titre (2.1) were within the accepted range of ≤15% CV for **Truenat® MTB** assay.

Cross reactivity: Study was performed to determine the cross reactivity of **Truenat® MTB** test to check for potential false positive results arising from cross-reactivity with other organisms unrelated to MTB. Cross reacting organisms from below listed ATCC and Zeptometrix were used in the study. Both negative and positive sputum samples were spiked with concentrations of 10^6 CFU/mL of bacteria, 10^6 $TCID_{50}$/mL of viruses and 10^6 copies/mL of nucleic acids, as applicable to the organisms. Both the MTB negative and positive samples were subjected to sample preparation on **Trueprep® AUTO** Universal Cartridge Based Sample Prep Device followed by PCR on **Truenat® MTB** test in triplicates runs.

Mycobacterium terrae (ATCC® 25149™)	*Mycobacterium scrofulaceum*(ATCC® 35788™)
Mycobacterium triviale (ATCC® 23290™)	*Mycobacterium avium* (ATCC® 700898™)
Mycobacterium gordonae (ATCC® 35759™)	*Mycobacterium chelonae* (ATCC® 35749™)
Mycobacterium xenopi (ATCC® 19974™)	*Mycobacterium fortuitum* (ATCC® 35931™)
Mycobacterium smegmatis(ATCC® 11759™)	*Mycobacterium intracellulare* (ATCC® 35772™)
Mycobacterium phlei(ATCC® 35784™)	*Streptococcus pneumoniae* (ATCC® BAA-334D-5™)
Mycobacterium abscessus (ATCC® 23003™)	*Haemophilus influenzae* (ATCC® 51907DQ™)
Escherichia coli (ATCC® BAA-2340™)	*Staphylococcus epidermidis* (Zeptometrix MRSE; RP62A)
Influenza A (Zeptometrix Taiwan/42/06)	Influenza B (Zeptometrix Yamagata/16/88)
Staphylococcus aureus (ATCC® 25923™)	*Nocardia* (ATCC® 12288™)
Pseudomonas aeruginosa (ATCC® BAA-47™)	*Mycobacterium kansasii* (ATCC® 12479™)
Mycobacterium marinum (ATCC® 29254™)	

The results showed no cross-reactivity of **Truenat® MTB** test with any of the above listed organisms. Hence, the assay was specific to MTB.

Stability of clinical samples during freeze-thaw: A study was conducted to demonstrate Stability of samples after Freeze Thaw for **Truenat® MTB** test. Stability of samples was tested by conducting multiple freeze-thaw cycles on three sputum samples spiked with 1000, 500 and 250 copies/mL of confirmed MTB positive specimens. The samples were frozen at -20°C and subjected to 5 freeze-thaw cycles. The samples were processed on **Trueprep AUTO** Universal Cartridge Based Sample Prep Device followed by PCR on **Truenat® MTB** test to determine the effect of Freeze-thaw process on the stability of MTB samples. The study concluded that the MTB samples were stable till the 3rd freeze-thaw cycle.

Accuracy:
Accuracy was determined by performing DNA extractions and **Truenat® MTB** PCR for varying titres of samples over 5 consecutive days. The standard deviation values obtained were within the accepted range of ≤1.5 Ct.

Clinical Validations:
a) Clinical validation 1: A pilot study was conducted at P. D. Hinduja National Hospital and Medical Research Centre (Nikam, Chaitali, et al. "Rapid diagnosis of *Mycobacterium tuberculosis* with **Truenat® MTB**: a near-care approach." PLOS One 8.1 (2013): e51121). 226 sputum specimens from suspected TB patients were analyzed using smear microscopy, culture, in-house nested PCR and **Truenat® MTB**. Pelleted sputum specimens were re-suspended in lysis buffer from the **Trueprep® MAG Sputum** kit and processed using the **Trueprep® MAG Sample Prep Device** followed by PCR on **Truenat® MTB** Chip-based Real Time PCR test. Results were compared with a Composite Reference Standard (CRS) comprising microbiological tests, clinical and radiological findings and patient history. The results are tabulated below:

	Smear		Culture		In-house Nested PCR		**Truenat® MTB**	
	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
CRS +ve	120	71	141	50	173	18	174	17
CRS -ve	00	35	00	35	03	32	00	35
Sensitivity %	62.83		73.82		90.58		91.10	
Specificity %	100		100		91.43		100	
PPV%	100		100		98.30		100	
NPV%	33.02		41.18		64.00		67.31	

CRS - Composite Reference Standard, PPV - Positive Predictive Value, NPV - Negative Predictive Value.
The results show that **Truenat® MTB** was the most sensitive (91.10%) and specific (100%) test compared with the Composite Reference Standard. **Truenat® MTB** also showed high sensitivities of 99.12% among smear positive and culture positive specimen and 75.86% among smear negative and culture positive specimen.
Another study evaluating the **Truenat® MTB** test was performed using a characterized 100 sample panel from suspected TB patients referred to a hospital in South East Asia. The study involved processing of 500µl of each sputum specimen using the **Trueprep® MAG Sputum** Sample Prep Kit on the **Trueprep® MAG Sample Prep Device**. The purified nucleic acids were tested using **Truenat® MTB** Chip-based Real Time PCR test and MTB specific primers and probe on a commercial real-time PCR machine.

Sample Type	Commercial real-time PCR machine result	**Truenat® MTB**
S+C+	40/40 (100%)	40/40 (100%)
S-C+	30/40 (75%)	30/40 (75%)
S-C-	0/20 (nil detected)	0/20 (nil detected)

(S: Smear, C: Culture)
The **Truenat® MTB** Chip-based Real Time PCR test was able to detect 100% of the S+C+ samples (40/40), 75% of S-C+ samples (30/40) and gave a negative result for 100% of the S-C- samples (20/20).

b) Clinical validation 2: validation of EPTB Samples
A retrospective study was conducted at P. D. Hinduja National Hospital and Medical Research Centre, Mumbai. A total of 266 specimens from suspected EPTB patients were analyzed using culture and **Truenat® MTB**. EPTB Specimens (Pleural / Peritoneal fluid, lymph node aspirate, Abscess, Bronchoalveolar lavage, Biopsy and Tissues) obtained from patients were processed using **Trueprep® MAG Sample Prep Device** followed by PCR on **Truelab® Uno** real time micro PCR analyzer using **Truenat® MTB** Chip-based Real Time PCR test. Sensitivity and specificity for each sample type was calculated taking Culture as 'Gold Standard'. The results are tabulated below:

Sample type/No.of Samples	BAL (n=27)	Pleural/ Peritoneal Fluid (n=57)	Abscess (n=81)	Biopsy (n=49)	Lymph node (n=32)	Tissue (n=20)
Sensitivity	84.62%	44.44%	82.22%	100%	71.43%	83.33%
Specificity	71.43%	89.58%	69.44%	93.33%	77.78%	92.86%

Sensitivity and specificity for all sample types combined was calculated against culture and found to be 78.00% and 84.07% respectively. The results are tabulated below:

Overall (All Sample Types)		
	Culture +Ve	Culture -Ve
Truenat MTB +Ve	71	28
Truenat MTB -Ve	20	147

c) Clinical validation 3:
A panel of 30 samples comprising of 10 known positives and 20 known negative sputum samples were tested on three different manufacturing lots of **Truenat® MTB** assay at National Institute for Research in Tuberculosis, Chennai against WHO approved system as comparator. DNA from 30 sputum samples were extracted using **Trueprep® AUTO** Universal Cartridge Based Sample Prep Device. The elutes were run in parallel on three lots of **Truenat® MTB** chips.
Specificity: All 20 negative samples by comparator assay were also found to be negative by the three lots of **Truenat® MTB** assay, showing **100%** specificity.
Sensitivity: All 10 positive sample results were correlated between the method giving a sensitivity of **100%** for the three lots of **Truenat® MTB** assay.
Concordance: The results obtained by **Truenat® MTB** assay on the10 positive samples were compared with the results obtained by the comparator assay. This showed good performance and 100% concordance with the WHO approved comparator test for all three lots on the tested panel of samples. Mean Standard deviation of Ct values across the 3 lots for MTB target was 0.45 well within acceptable Ct variation of 1.66 indicates (0.5 log) showed good performance and no significant lot to lot variation.

d) Clinical validation 4:
A study evaluating the performance of **Truenat® MTB** assay was conducted at National Reference Laboratory of Mycobacteriology, Tunisia. A total of 114 specimens (91 sputum and 23 extrapulmonary specimens) were collected from suspected TB patients were analysed. Out of 91 sputum samples, 25 sputum specimens from patients with other lung infections were taken as negative controls. All collected specimens were analysed using smear microscopy, culture, comparator PCR assay and **Truenat® MTB** and **Truenat® MTB-RIF** assay. Results were compared with a composite reference standard (CRS) comprising microbiological test results, clinical and radiological findings and patient history to assess the performance of the **Truenat® MTB** assay.
Performance (% of cases detected) of molecular tests in various specimen categories:

Test	S+ (N=46)	C+ (N=49)	S+C+ (N=46)	S-C+ (N=3)
Comparator assay	100	95.9	97.8	66.6
Truenat MTB	100	100	100	100

(S: Smear, C: Culture)
Sensitivity: The **Truenat® MTB** Chip-based Real Time PCR Test was able to detect all of the S+C+ specimens (46/46) and S-C+ specimens (3/3), showing **100%** sensitivity.
Specificity: All 48 CRS negative specimens were also found to be negative by **Truenat® MTB** assay, showing **100%** specificity.
Rifampicin Resistance: 4 strains are resistant to rifampicin by proportion method and comparator assay, 3 strains are resistant by **Truenat® MTB-RIF** and for one strain, the test is indeterminate as quantity of specimen was not enough.

e) Clinical validation 5:
A multicentric prospective clinical evaluation study was performed by FIND(Foundation For Innovative New Diagnostics) in 19 clinical sites and 7 reference laboratories in 4 countries (India, Peru, Ethiopia, Papua New Guinea) to determine the diagnostic accuracy of the Truenat assays. In this study samples were collected from 1,654 participants. The study was performed in microscopy centers which were the intended use settings. Culture was used as the reference standard. The performance of the **Truenat® MTB** was also compared head-to-head (on the same specimens) to Xpert or Ultra in reference laboratories. All sites performed Xpert, apart from sites in Peru, which performed Ultra.
Comparison with Microscopy: Overall, for sputum tested in microscopy centers, **Truenat® MTB** test showed a sensitivity of 73.3% and the specificity of **Truenat® MTB** test was 97.9%. The results are summarized in Table.1.

Table 1 : Performance of Truenat® MTB at the microscopy centre				
Test	**Sensitivity% (95% CI)**	**Sensitivity % Smear Pos (95% CI)-N**	**Sensitivity % Smear Neg (95% CI)-N**	**Specificity % (95% CI)**
Truenat® MTB	73.3 [67.5,78.3]	90.8 [85.6,94.3]-N:174	36.9 [27.4,47.6]-N:84	97.9 [96.8,98.6]

Comparison with Xpert: Truenat® MTB test showed a sensitivity of 83.5% and specificity of 97.4% while, Xpert gave a sensitivity of 85.3% and specificity of 97.1% respectively. The results are summarized in Table.2.

Table 2: Performance of Truenat® MTB in comparison with Xpert				
Test	**Sensitivity% (95% CI)**	**Sensitivity % Smear Pos (95% CI)-N**	**Sensitivity % Smear Neg (95% CI)-N**	**Specificity % (95% CI)**
Xpert	85.3[80.0, 89.3]	98.8 [95.7,99.7]-N:164	48.3 [36.2,60.7]-N:60	97.1 [95.7,98.0]
Truenat® MTB	83.5 [78.1,87.8]	97.6 [93.9,99.1]-N:164	45 [33.1,57.5]-N:60	97.4 [96.1,98.3]

Comparison with Ultra: Truenat® MTB test showed a sensitivity of 72.8% and specificity of 99.3% while, Ultra gave a sensitivity of 95.7% and specificity of 97.2% respectively. The results are summarized in Table.3.

Table 3: Performance of Truenat® MTB in comparison with Ultra				
Test	**Sensitivity% (95% CI)**	**Sensitivity % Smear Pos (95% CI)-N**	**Sensitivity % Smear Neg (95% CI)-N**	**Specificity % (95% CI)**
Ultra	95.7 [89.3,98.3]	100 [93.0,100.0]-N:51	90.2 [77.5,96.1]-N:41	97.2 [94.6,98.6]
Truenat® MTB	72.8 [63.0,80.9]	94.1 [84.1,98.0]-N:51	46.3 [32.1,61.3]-N:41	99.3 [97.5,99.8]

19. REFERENCES

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SYMBOL KEYS

Consult instructions for use	IVD *In vitro* Diagnostic Medical Device. Not for medicinal use.	Temperature Limitation	REF Catalogue Number	For single use only	This Way Up	Manufacturer	UDI Unique Device Identifier
Date of Manufacture	Date of Expiry	LOT Batch Number / Lot Number	Caution	Contains sufficient for <n> tests	Keep dry	Keep away from sunlight	EC REP Authorised Representative in European Community

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