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# Truenat®

## MTB Plus

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

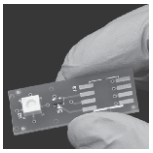
### 1. INTENDED USE

**Truenat® MTB Plus (REF601130005 / 601130020 / 601130025 / 601130050 / 601130100 / 601130200)** is an automated point-of-care or near patient Chip-based Real Time Polymerase Chain Reaction (PCR) test for the semi-quantitative detection and diagnosis of *Mycobacterium tuberculosis* (MTB) in human pulmonary (sputum/non-sputum) and EPTB specimen and aids in the diagnosis of infection with MTB. **Truenat® MTB Plus** runs on the **Truelab®** Real Time Quantitative micro PCR Analyzers. **Truenat® MTB Plus** 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 micro PCR System enables decentralization and near patient diagnosis of *Mycobacterium tuberculosis* 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 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 Kits 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 Plus** 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 Plus** 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.**

### 3. PRINCIPLE OF THE TEST

**Truenat® MTB Plus** works on the principle of Real Time Polymerase Chain Reaction. 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 Plus** 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 Plus** chip and the test is started. A positive amplification causes the dual labeled fluorescent probe in the **Truenat® MTB Plus** 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, a semi-quantitative result 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 20000 results can be stored in the **Truelab® Uno Dx/Duo/Quattro** analyzer for future recall and reference.

### 4. TARGET SELECTION

The target sequences for this kit are *nrdz* gene (that codes for ribonucleoside-diphosphate reductase adenosyl cobalamin-dependent protein) and IS6110 gene sequence. The regions selected are specific to the MTB complex.

### 5. CONTENTS OF THE Truenat® MTB Plus KIT

A. Individually sealed pouches
B. Package Insert
   Each individually sealed pouch contains:
   1. **Truenat® MTB Plus** 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 | 601130005 | 601130020 | 601130025 | 601130050 | 601130100 | 601130200 |
|---|---|---|---|---|---|---|
| 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 Plus** micro PCR chip is stable for two (2) years from the date of manufacture if stored between 2-30°C. It is stable for upto 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** 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), **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 Plus** 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/AUTO v2** 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 Plus** 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 Quantitative micro PCR Analyzer user manual)

1. Switch on the **Truelab®** Analyzer.
2. Select user and enter password.
3. For **Truelab® Uno Dx**, select the test profile for "MTB Plus" 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 Plus" 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 Plus** 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 Plus** 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 Plus** 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 Plus** 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 Plus** 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 MTB load as "HIGH (Ct<20)","MEDIUM (20≤Ct<25)", "LOW (25≤Ct<30)" or " VERY LOW (Ct ≥ 30)" 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 Plus** 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®** Real Time Quantitative micro PCR 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 Plus** chip, microtube, microtube cap, pipette tips, lysis buffer tube 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.

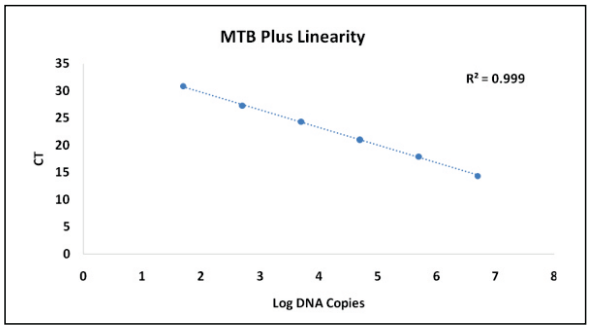
## 18. SPECIFIC PERFORMANCE CHARACTERISTICS

**Analytical exclusivity (Primer Specificity):**

The following viruses and 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 Plus** assay and found not to interfere in assay performance.

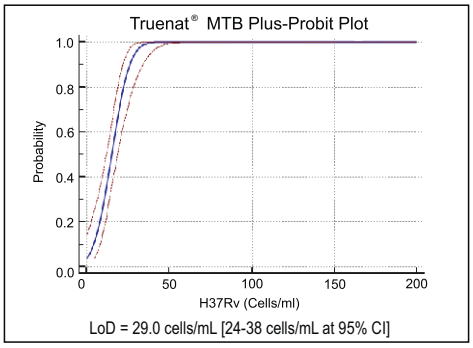
| Bacteria | Bacteria | Virus |
|---|---|---|
| *Yersenia enterocolitica* | *Acinetobacter anitratus* | Adenovirus |
| *Trichomonas vaginalis* | *Candida albicans* | Cytomegalovirus |
| *Enterococcus faecalis* | *Chlamydia trachomatis* | Hepatitis B virus |
| *Pseudomonas trivialis* | *Enterobacter cloacae* | Hepatitis C Virus |
| *Gardeneralla vaginalis* | *Salmonella enterica* | Human Immunodeficiency Virus |
| *Klebsiella pneumoniae* | *Staphylococcus aureus* | Epstein-Barr virus |
| *Streptococcus mutans* | *Escherichia coli* | Herpes Simplex virus |
| | | Simian virus |
| | | Human Papilloma Virus |

**Linearity:** Serial dilutions of Zepto *Mycobacterium tuberculosis* H37Rv were made from 5.00E+06 to 5.00E+01 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 Plus** test is found to be linear over 6 orders of magnitude (from 5.00E+06 to 5.00E+01 copies/mL) for Zepto *Mycobacterium tuberculosis* H37Rv.




**Limit of detection (LoD)**:

LoD was determined by testing the dilutions (500 cells/ml, 300 cells/ml, 200 cells/ml, 100 cells/ml, 50 cells/ml, 25 cells/ml, 12.5 cells/ml, 0 cells/ml) of H37Rv cells [Zeptometrix, Lot:319177]. Each dilution was extracted on **Trueprep® AUTO** Universal Cartridge Based Sample Prep Device with 50 µl of H37Rv spiked in 450 µl negative sputum for 24 times followed by PCR on **Truelab® Uno Dx** Real Time micro PCR Analyzer for respective dilution. LoD was found to be 29.0 cells/mL of sputum for **Truenat® MTB Plus** assay.



LoD = 29.0 cells/mL [24-38 cells/mL at 95% CI]

**Robustness:**

To determine whether the **Truenat® MTB Plus** Chip-based Real Time PCR test showed any signs of carryover of PCR products between 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 Plus** 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 Plus** 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 MTB Plus as Inter User (0.71), Inter day (1.15) and Inter Device (1.40) which were in the accepted range of ≤15% CV for **Truenat® MTB Plus** assay.

**Precision:**

Precision was tested by performing **Truenat® MTB Plus** assay with extracted DNA of High, Medium and Low titres for MTB Plus for five consecutive days. Every day PCR for each titre DNA was run in duplicates. The % CV values obtained for High titre (1.85), Medium titre (3.08) and low titre (1.86) for MTB Plus were within the accepted range of ≤15% CV for **Truenat® MTB Plus** assay.

**Accuracy:**

Accuracy was determined by performing DNA extractions and **Truenat® MTB Plus** PCR for varying titres of samples over 5 consecutive days. The CV values obtained were within the accepted range of ≤15% for **Truenat® MTB Plus** assay.

**Clinical validation 1:**

A panel of 30 samples comprising of 10 known positives and 20 known negative sputum samples were tested on three different lots of **Truenat® MTB Plus** 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 test lots of **Truenat® MTB Plus** chips.

**Specificity:** All 20 negative samples by comparator assay were also found to be negative by the three lots of **Truenat® MTB Plus** 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 Plus** assay.

**Concordance:** The results obtained by **Truenat® MTB Plus** assay showed 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 0.79, well within acceptable Ct variation of 1.66 indicates (0.5 log) showed good performance and no variation was seen between the three lots.

**Clinical validation 2:**

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 Plus** 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: Truenat® MTB Plus** test gave a sensitivity of 79.8% and specificity of 96.5%. The results are summarized in Table.1.

**Table 1 : Performance of Truenat® MTB Plus 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 Plus | 79.8 [74.5,84.3] | 96 [91.9,98.0]-N:174 | 46.4 [36.1,57.0]-N:84 | 96.5 [95.2,97.4] |

**Comparison with Xpert: Truenat® MTB Plus** test showed a sensitivity of 87.1% and specificity of 95.4% while, Xpert gave a sensitivity of 85.3% and specificity of 97.1%. The results are summarized in Table.2.

**Table 2: Performance of Truenat® MTB Plus 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 Plus | 87.1 [82.0,90.8] | 98.8 [95.7,99.7]-N:164 | 55 [42.5,66.9]-N:60 | 95.4 [93.8,96.6] |

**Comparison with Ultra: Truenat® MTB Plus** test showed a sensitivity of 79.3% and specificity of 97.5% while, Ultra gave a sensitivity of 95.7% and specificity of 97.2%. The results are summarized in Table.3.

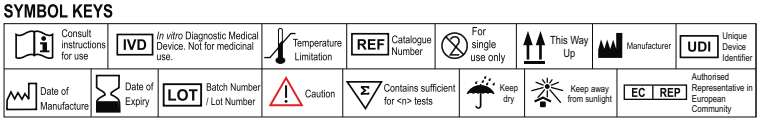
**Table 3: Performance of Truenat® MTB Plus in comparison with Ultra**

| 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 Plus | 79.3 [70.0,86.4] | 96.1 [86.8,98.9]-N:51 | 58.5 [43.4,72.2]-N:41 | 97.5 [95.0,98.8] |

## 19. REFERENCES


1. WHO Fact sheet March 2012. http://www.who.int/mediacentre/factsheets/fs104/en/.
2. Todar's Online Textbook of Bacteriology - Kenneth Todar, Ph.D.
3. WHO report 2011 Global Tuberculosis Control.
4. Karen R Steingart et. al. (2006) Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: a systematic review. The Lancet Infectious Diseases - Volume 6, Issue 10, pp 664 - 674.
5. P. Farnia et. al. (2002) Improving Sensitivity of Direct Microscopy for Detection of Acid-Fast Bacilli in Sputum: Use of Chitin in Mucus Digestion, J. Clin Microbiol.; 40(2): 508-511.
6. E. Ogbaini-Emovon (2009) Current Trends In The Laboratory Diagnosis of Tuberculosis, Benin Journal of Post Graduate Medicine, Vol.11 Supplemental, pp. 79 - 90.
7. Dye C, Watt C.J., Bleed D.M. et. al. (2005). Evolution of tuberculosis control and prospects for reducing tuberculosis incidence, prevalence, and deaths globally. JAMA 2005; 293: 2767-2775.
8. N. Kennedy et. al (1994) Polymerase Chain Reaction for Assessing Treatment Response in Patients with Pulmonary Tuberculosis. The Journal of Infectious Diseases Vol. 170, No. 3, pp.713-716.
9. WHO consolidated guidelines on tuberculosis(2020). Module 3: Diagnosis, Rapid diagnostics for tuberculosis detection


**SYMBOL KEYS**





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**Molbio Diagnostics Private Limited**

**Registered Office & Manufacturing Unit - I:**
Plot No. L-46, Phase II D, Verna Industrial Estate, Verna, Goa - 403 722, INDIA
**Manufacturing Unit - II:**
Plot No. L-42, Phase II B, Verna Industrial Estate, Verna, Goa - 403 722, INDIA
www.molbiodiagnostics.com
Email: sales@molbiodiagnostics.com (Sales Enquiries)
customersupport@molbiodiagnostics.com (Feedback and Customer Support)
EC REP Qarad EC-REP BV, Pas 257, 2440 Geel, Belgium


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