CE

Truenat™

MTB Plus

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

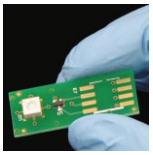
1. INTENDED USE

Truenat™ MTB Plus (REF 601130005 / 601130020) is a chip-based Real Time Polymerase Chain Reaction (PCR) test for the semiquantitative 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.

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 micro PCR Analyzer and **Trueprep™ MAG/AUTO** Sample Prep Device and room temperature stable **Truenat™** micro PCR chip and **Trueprep™ MAG/AUTO** 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 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™ Uno / Truelab™ Uno Dx / Truelab™ Duo / Truelab™ Quattro / Trueprep™ AUTO / Trueprep™ MAG / Truepet™ / Truenat™ are all registered trademarks of Molbio Diagnostics (P) Limited.

The Truelab™ Real Time micro PCR Analyzer is protected by the following patents and patents pending: IN 2313/CHE/2007, WO 2009/047804 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™ MAG** Sample Prep Device and **Trueprep™ MAG** Sputum Sample Prep kit or using **Trueprep™ AUTO** Universal Cartridge Based Sample Prep Device and **Trueprep™ AUTO** Universal Cartridge Based Sample Prep Kit. The **Truenat™ MTB Plus** chip is placed on the chip tray of the **Truelab™** Real Time 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 5000 results in **Truelab™ Uno** to 20000 results in **Truelab™ Uno Dx/Duo/Quattro** can be stored on the analyzer for future recall and reference.

4. TARGET SELECTION

The target sequences for this kit are part of the ribonucleoside-diphosphate reductase gene, the product of which provides the precursor for DNA synthesis and the IS6110 sequence. The regions selected are specific to the MTB complex.

5. CONTENTS OF THE Truenat™ MTB Plus KIT

A. Individually sealed pouches, each containing
1. **Truenat™ MTB Plus** micro PCR chip.
2. Microtube with freeze dried PCR reagents.
3. DNase & RNase free pipette tip.
4. Desiccant pouch.

B. Package Insert.

REF	601130005	601130020
Σ	5T	20T

6. CONTENTS OF Trueprep™ AUTO MTB Sample pre-treatment pack (only for Trueprep™ AUTO users)

A. Liquefaction buffer
B. Lysis buffer
C. Disposable transfer pipette (graduated)

REF	60204AS05	60204AS20
Σ	5T	20T

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 also stable for upto six (6) months at temperatures up to 40°C and 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 603010001/62301001/ 633010001/64301001) consisting of,

1. **Trueprep™ MAG / Trueprep™ AUTO** Sample Prep Device (REF 603040001/ 603041001).
2. **Truelab™ Uno / Truelab™ Uno Dx / Truelab™ Duo / Truelab™ Quattro** Real Time micro PCR Analyzer (REF603020001/603021001/603022001/ 603023001).
3. **Truelab™** micro PCR Printer (REF 603050001).
4. **Truepet™** SP fixed volume precision micropipette - 6 µl (REF 604060006).
5. **Truelab™** Microtube Stand (REF 603070001).

Also required additionally are: **Trueprep™ MAG Sputum** Sample Prep Kit (REF602020005/REF 602020050) / **Trueprep™ AUTO** Universal Cartridge Based Sample Prep Kit (REF60203AR05/REF 60203AR25), **Truenat™** Universal Control Kit (REF 601100008), DNase and RNase-free pipette tips with filter barrier, which may also be procured from **Molbio**, Powder free disposable latex gloves, waste disposal container with lid.

9. SPECIMEN PREPARATION FOR EXTRACTION WITH Trueprep™ MAG

Truenat™ MTB Plus requires purified nucleic acids from pulmonary (sputum/non-sputum) and EPTB specimen that are extracted using the **Trueprep™ MAG** Sample Prep Device and **Trueprep™ MAG** Sputum Sample Prep Kit (Refer to the User Manual of **Trueprep™ MAG** Sample Prep Device and the package insert of **Trueprep™ MAG** Sputum Sample Prep Kit for details).

10. SPECIMEN PREPARATION FOR EXTRACTION WITH Trueprep™ AUTO

Truenat™ MTB Plus requires purified nucleic acids from pulmonary (sputum/non-sputum) and EPTB specimen that are extracted using the **Trueprep™ AUTO** Universal Cartridge Based Sample Prep Device and **Trueprep™ AUTO** Universal Cartridge Based Sample Prep Kit. Samples must be liquefied and pre-treated using the **Trueprep™ AUTO MTB** sample pre-treatment pack provided, as per protocol below, before proceeding for extraction.

For sputum samples

Check if the specimen is pipettable. If not, add 1 drop of liquefaction buffer to the specimen (If specimen is frozen allow it to reach room temperature first). Allow the reagent to hydrate the sample by swirling gently. Incubate at room temperature for 5 minutes. If sample has not liquefied after 5 minutes, incubate for another 5 minutes until sample is pipettable. This depends on sample viscosity and ambient temperature.

Label a lysis buffer bottle with patient ID and transfer 500 µl of the liquefied sample into the lysis buffer bottle using the graduated disposable transfer pipette provided. Add 2 drops of the liquefaction buffer to the lysis buffer bottle and mix gently. Close the cap tightly and mix well. Wait for 3 minutes.

Check if contents are fully liquefied by shaking the bottle. If not, incubate it further till the contents are liquefied. Depending upon the sample this may take another 10 to 15 minutes. Do not proceed if the content has not liquefied. Dispose off lysis buffer tube after use, as per the section on "Disposal and Destruction" (Section 18).

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** Universal Cartridge Based Sample Prep Kit package insert.(Refer to the User Manual of **Trueprep™ AUTO** Universal Cartridge Based Sample Prep Device and the package insert of **Trueprep™ AUTO** Universal Cartridge Based Sample Prep Kit for details).

For non- sputum samples

Refer protocol sheet in the **Trueprep™AUTO** MTB Sample Pre-Treatment pack.

Nucleic acid extraction: Follow Extraction procedure (section-13) of **Trueprep™ AUTO** Universal Cartridge Based Sample Prep Kit package insert.(Refer to the User Manual of **Trueprep™ AUTO** Universal Cartridge Based Sample Prep Device and the package insert of **Trueprep™ AUTO** Universal Cartridge Based Sample Prep Kit for details).

11. 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 and package inserts of all the components of the **Truelab™ Real Time micro PCR System** before use.
5. All materials of human origin should be handled as though potentially infectious.
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.

12. 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.

13. 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.

14. 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.

15. TEST PROCEDURE

(Please also refer the **Truelab™** Real Time micro PCR Analyzer user manual)

1. Switch on the **Truelab™** Analyzer.
2. If using the **Truelab™ Uno** device, also switch on the touch screen. If using the **Truelab™ Uno Dx/Duo/Quattro**, proceed to step 3.
3. Select user and enter password.
4. For **Truelab™ Uno/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 (Idle1/2) for **Duo** and (Idle1/2/3/4) 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.
5. Enter the patient details as prompted in the **Truelab™** Analyzer screen.
6. Press Start Reaction.
7. For **Truelab™ Uno/Uno Dx,** Press the eject button to open the chip tray. For **Truelab™ Duo/Quattro**, the chip tray opens automatically on tapping the "Start Reaction" button.
8. Open a pouch of **Truenat™ MTB Plus** and retrieve the micro PCR chip, microtube and the DNase & RNase free pipette tip.
9. Label the chip with the patient ID using a marker pen at the space provided on the back side of the chip.
10. Place the **Truenat™ MTB Plus** chip-based Real Time PCR test on the chip tray without touching the white reaction well. The reaction well should be facing up and away from the Analyzer. Gently press the chip to ensure that it has seated in the chip tray properly.
11. Place the microtube containing freeze dried PCR reagents in the microtube stand provided along with the **Truelab™** Real Time micro PCR workstation **after ensure that white pellet of dried PCR reagents remain at the bottom of the microtube.** Remove the microtube cap and dispose it off as per the section on "Disposal and Destruction" (Section 18). Using the filter barrier tip provided in the pouch, pipette out six (6) µL of the purified DNA from the Elute Collected Tube into the microtube. Allow it to stand for 30-60 seconds 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 18).
12. For **Truelab™ Uno/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 Done on the "Please Load Sample" Alert message. For **Truelab™ Duo/Quattro**, select "YES" at the Please load Sample prompt. Chip tray will close automatically and the reaction will start.
13. Read the result from the screen.
14. After the reaction is completed, for **Truelab™ Uno/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.
15. 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 18).
16. 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).
17. Switch off the **Truelab™** Analyzer.

16. 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 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", "MEDIUM", "LOW" or "VERY LOW" 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.

17. QUALITY CONTROL PROCEDURES

To ensure that the **Truelab™** Real Time micro PCR Analyzer is working accurately, run positive and negative controls from time to time. The **Truenat™** Universal Control Kit 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.

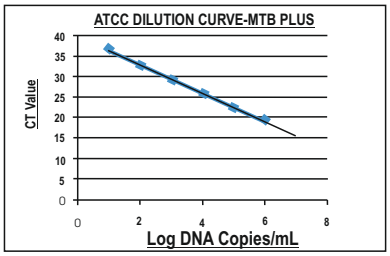
18. DISPOSAL AND DESTRUCTION

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 contaminated fluid or 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.

19. SPECIFIC PERFORMANCE CHARACTERISTICS

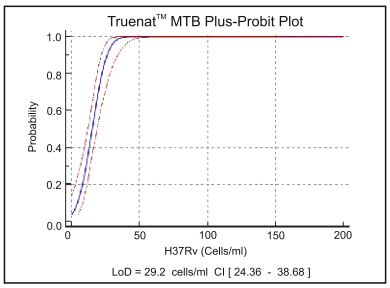
Assay range and Limit of detection:

Linearity: Assay shows linearity over clinical testing range. Below graph shows linearity over 6 orders of magnitude [$R^2 = 0.995$].



Limit of detection (LOD):

LOD is defined as concentration that gives 95% Positivity using Probit analysis. Serial dilutions were done using quantified H37Rv cells [Zeptometrix, Lot:319177].24 replicate testing of each dilution was performed. 95% positivity was obtained at 29.2 cells/ml concentration.



Primer Specificity

The following viruses and microorganisms were evaluated *in silico* and *in vitro* for potential cross-reactivity in the **Truenat™ MTB Plus** assay and found not to interfere in assay performance.

Bacteria (NTM)	Bacteria	Virus
M. malmoense	*Acinetobacter anitratus*	Adenovirus
M. intracellulare	*Candida albicans*	Cytomegalovirus
M. scrofulaeium	*Chlamydia trachomatis*	Hepatitis B virus
M. ulcerance	*Enterobacter cloacae*	Hepatitis C Virus
M. abscessus	*Salmonella enterica*	Human Immunodeficiency Virus
M.fortuitum	*Staphylococcus aureus*	Epstein-Barr virus
M. avium	*Streptococcus mutans*	Herpes Simplex virus
M.gordanae	*Escherichia coli*	Simian virus
M.szulgai	*Gardeneralla vaginalis*	
M.kansasii	*Neisseria gonorrheae*	
Trichomonas vaginalis	*Enterococcus faecalis*	

20. REFERENCES

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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.
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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.

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 Side Up	Manufacturer	
Date of Manufacture	Date of Expiry	LOT Batch Number / Lot Number	Caution	Σ Contains sufficient for <n> tests	EC REP Authorised Representative in the European Community		

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