

M. leprae

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

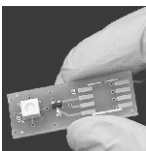
1. INTENDED USE

REF Truenat[®] M. leprae (REF 601700005 / 601700020 / 601700025 / 601700050 / 601700100 / 601700200) is an automated point-of-care or near patient Chip-based Real Time Polymerase Chain Reaction (PCR) test for the semi-quantitative detection of *Mycobacterium leprae* in slit skin smear specimens and aids in the diagnosis of patients with leprosy. Truenat[®] M. leprae runs on the Truelab[®] Real Time Quantitative micro PCR Analyzer. Truenat[®] M. leprae 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

Leprosy is a chronic infectious disease of skin and peripheral nerves caused by *Mycobacterium leprae* and are of special concern because it can progress to peripheral neuropathy and permanent progressive deformity. *Mycobacterium leprae* is an obligate intercellular pathogen, has existed throughout recorded history. Leprosy is a chronic infectious disease of skin, nasal cavity and peripheral nerves which eventually leads to disability, disfigurement and socioeconomic problems. Although multidrug has been very successful in reducing the prevalence of leprosy by 90% in the last 3 decades, but the annual incidence has not yet declined in most countries where the disease is highly endemic, with >200000 new cases annually. According to the clinical spectrum proposed by Ridley and Jopling leprosy is characterized by two polar forms, a paucibacillary tuberculoid and a multibacillary lepromatous form and by three intermediate forms borderline tuberculoid, borderline and borderline lepromatous forms. Patients can also exhibit a rare form known as pure neural leprosy, which is an isolated clinical entity characterized mainly by the uniqueness of nerve lesions. Most people within population where leprosy is endemic have been exposed to *M. leprae*, but few develop the disease, suggesting that a minority are susceptible to leprosy while the majority of the population present some sort of genetic resistance. Standard immunological and histological approaches for disease assessment are less effective in the diagnosis of early leprosy, therefore disease and transmission can progress. The *in vitro* assays such as those based on radiorespirometry and the Live/Dead BacLight fluorescent viability assay require large quantities of bacteria, (i.e., 10⁷ and 10⁸ bacteria) respectively, for reliable detection and are therefore not applicable for direct detection in clinical specimens. The accurate diagnosis of leprosy is of fundamental importance to all aspects of leprosy epidemiology, case management and prevention of disability. The development of polymerase chain reaction (PCR) has brought an unprecedented opportunity for sensitive, specific and rapid detection of *M. leprae* in clinical specimens.

The Truenat[®] point-of-care real time PCR system enables decentralization and near patient diagnosis and detection of *M. leprae* by making real time PCR technology rapid, simple, robust and user friendly, offering "sample to result" capability even at resource limited settings. This is achieved through a combination of lightweight, portable, mains / battery operated Truelab[®] Real Time Quantitative micro PCR Analyzer and Trueprep[®] AUTO/AUTO v2 Universal Cartridge Based Sample Prep Device and room temperature stable Truenat[®] micro PCR chips and Trueprep[®] AUTO/AUTO v2 Universal Cartridge Based Sample Prep Kits so that even the peripheral laboratories with minimal infrastructure and minimally trained technician 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[®] M. leprae is a disposable, room temperature stable, micro PCR chip with dried MgCl₂ in reaction well and freeze dried PCR reagents in microtube for performing Real Time PCR test for *Mycobacterium leprae* and runs on the Truelab[®] Real Time Quantitative micro PCR Analyzer. All components of Truenat[®] pouch are nuclease-free. It requires only six (6) µL of purified DNA to be added to the reaction well for the analysis. The intelligent chip also carries test and batch related information. The Truenat[®] M. leprae chip also stores information of used chips to prevent any accidental re-use of the chip.

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[®] M. leprae works on the principle of Real Time Polymerase Chain Reaction based on Taqman chemistry. The patient sample is first pre-treated using the Trueprep[®] AUTO Universal Sample Pre-treatment Pack. The DNA from the pre-treated sample is then extracted using Trueprep[®] AUTO/AUTO v2 Universal Cartridge Based Sample Prep Device and Trueprep[®] AUTO/AUTO v2 Universal Cartridge Based Sample Prep Kit. The cartridge from the Trueprep[®] AUTO/AUTO v2 Universal Cartridge Based Sample Prep Kit contains pre-loaded Internal Positive Control (IPC), composed of known concentration of DNA, trehalose, PBS buffer and amaranth dye, which is co-extracted along with sample nucleic acids, thereby validating the process from extraction to PCR run. The DNA extract is analyzed using the Truenat[®] M. leprae Chip-based Real Time Polymerase Chain Reaction (PCR) test and the Truelab[®] Real Time Quantitative micro PCR Analyzer. The Truenat[®] M. leprae 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 provided calibrated micropipette and tip and dispensed into the reaction well of the Truenat[®] M. leprae chip and the test is started. A positive amplification causes the dual labeled fluorescent probe in the Truenat[®] M. leprae chip 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, M. leprae "DETECTED" or "NOT DETECTED" result is displayed and in positive cases, semi-quantitative result is also displayed on the screen. Based on the detection 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 using the Truelab[®] micro PCR printer or transferred to the lab computer/or any remote computer via Wifi network or 4G/3G/GPRS network. Upto 20000 results in Truelab[®] Uno Dx/Duo/Quattro can be stored on the analyzer for future recall and reference.

4. TARGET SELECTION

The target sequence for the Truenat[®] M. leprae test is 16S ribosomal RNA gene, sodA gene and gene coding for RLEP region.

5. CONTENTS OF THE Truenat[®] M. leprae KIT

- Individually sealed pouches
- Package Insert

Each individually sealed pouch contains:

- Truenat[®] M. leprae micro PCR chip (1 No.)
- Microtube with freeze dried PCR reagents (1 No.)
- DNase and RNase free pipette tip (1 No.)
- Desiccant pouch (1 No.)

Pack sizes of Truenat[®] M. leprae

REF	601700005	601700020	601700025	601700050	601700100	601700200
	5T	20T	25T	50T	100T	200T

6. CONTENTS OF THE Trueprep[®] AUTO Universal Sample Pre-treatment Pack

- Lysis buffer.
- Disposable transfer pipette (graduated).
- Package insert.

Pack sizes of Trueprep[®] AUTO Universal Sample Pre-treatment Pack

REF	60205AB05	60205AB20	60205AB25	60205AB50	60205AB100	60205AB200
	5T	20T	25T	50T	100T	200T

7. CONTENTS OF THE Trueprep[®] AUTO/AUTO v2 Universal Cartridge Based Sample Prep Kit

A. The reagent pack contains the following reagents

No.	Contents	Purpose
1.	Wash Buffer A	To wash inhibitors from the sample
2.	Wash Buffer B	To wash inhibitors from the sample
3.	Elution Buffer	To elute nucleic acids
4.	Priming Waste	To purge residual liquid from tubing

B. The cartridge pack contains the following:

No.	Contents	Purpose
1.	Cartridge	Cartridges containing immobilized internal control (IPC) for extraction
2.	Elute collection tube (ECT)	Capped tubes for collection and storage of extracted nucleic acids
3.	Elute collection tube (ECT) label	To label Elute Collection Tube (ECT)
4.	Disposable transfer pipette	To pierce the seal of elute chamber and to transfer extracted nucleic acids from elute chamber of cartridge into the Elute Collection Tube (ECT)

C. Disposable transfer pipettes (graduated) - 3 mL

D. Reagent reset card-1 No.

E. Package insert

Pack sizes of **Trueprep® AUTO Universal Cartridge Based Sample Prep Kit**

REF	60203AR05	60203AR25	60203AR50	60203AR100	60203AR200
	5T	25T	50T	100T	200T

Pack sizes of **Trueprep® AUTO v2 Universal Cartridge Based Sample Prep Kit**

REF	60207AR05	60207AR25	60207AR50	60207AR100	60207AR200
	5T	25T	50T	100T	200T

8. STORAGE AND STABILITY

- Truenat® M. leprae** test is stable for two (2) years from the date of manufacture if stored between 2-30°C. It is also stable for one (1) month at temperatures up to 45°C. Avoid exposure to light or elevated temperatures (above recommended levels). Do not freeze.
- Trueprep® AUTO** Universal 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.
- Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit is stable for two (2) years from the date of manufacture if stored between 2°C to 40°C. It is also stable for one (1) month at temperatures up to 45°C. Avoid exposure to light.
- Do not open the pouch until ready to test. Make sure to start the test promptly after 30-60 seconds of adding the elute to the microtube.
- Do not use the pouch if torn.
- Do not use pouches that have passed the expiration date.

9. MATERIALS REQUIRED BUT NOT PROVIDED WITH THE KIT

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

- Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device (REF 603041001/603042001)
- Truelab® Uno Dx / Truelab® Duo / Truelab® Quattro** Real Time micro PCR Analyzer (REF 603021001/603022001/603023001).
- Truelab®** micro PCR Printer (REF 603050001).
- Truepet®** SPA fixed volume precision micropipette - 6 µl (REF 604070006).
- Truelab®** Microtube Stand (REF 603070001).

Also required additionally are: **Trueprep® AUTO** Universal Sample Pre-treatment Pack (REF 60205AB05 / 60205AB20 / 60205AB25 / 60205AB50 / 60205AB100 / 60205AB200), **Trueprep® AUTO** Universal Cartridge Based Sample Prep Kit (REF 60203AR05 / 60203AR25 / 60203AR50 / 60203AR100 / 60203AR200) or **Trueprep® AUTO v2** Universal Cartridge Based Sample Prep Kit (REF 60207AR05 / 60207AR25 / 60207AR50 / 60207AR100 / 60207AR200), powder free disposable gloves, waste disposal container with lid and sodium hypochlorite.

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

Truenat® M. leprae requires purified nucleic acids from slit skin smear specimens. Take 100mg of the Slit skin tissue specimen in a micro tube. To the tube, 100 µL of Lysis buffer from **Trueprep® AUTO** Universal Sample Pre-treatment Pack is added and tissue is homogenized with micro pestle. After homogenization the entire content is then transferred to the remaining lysis

buffer tube and it is allowed to stand for 5 minutes at roomtemperature. (Refer to the package insert of **Trueprep® AUTO** Universal Sample Pre-treatment Pack for further details).

Sample Storage and Transportation:

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

Nucleic acid extraction: Use entire content from the lysis buffer tube containing specimen for further procedure with the **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device and **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit (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 the lysis buffer tube and transfer pipette after use, as per the section on "Disposal and Destruction" (Section 18).

11. SAFETY PRECAUTIONS

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

12. PROCEDURAL PRECAUTIONS

- 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.
- Do not perform the test in the presence of reactive vapours (e.g. from sodium hypochlorite, acids, alkalis or aldehydes) or dust.
- While retrieving the **Truenat® M. leprae** micro PCR chip, microtube and the DNase and RNase free pipette tip from the pouch, ensure that neither bare hands nor gloves that have been used for previous tests are used.
- Ensure that the colour of the desiccant pouch is orange after opening a sealed **Truenat®** chip pouch. If the colour of the desiccant pouch changes from orange to white due to the absorption of moisture, do not use the contents of the **Truenat®** chip pouch.

13. PROCEDURAL LIMITATIONS

- Optimal performance of this test requires appropriate specimen collection, handling, storage and transport to the test site.
- 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.
- 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.
- 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

- 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.
- Sodium hypochlorite should not be used on an acid-containing spill unless the spill-area is wiped dry first. Materials used to clean spills, including

gloves should be disposed off as potentially bio-hazardous waste e.g. in a bio-hazard waste container.

15. 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 “M-Leprae” to be run from the Profiles Screen on the analyzer screen. For **Truelab**® **Duo/Quattro**, select the Bay (I/II) for **Duo** and (III/III/IV) for **Quattro** from the Status Screen to view the Profiles Screen. Select the test profile for “M-Leprae” to be run from the Profiles Screen, on the analyzer screen.
4. Enter the patient details as prompted in the **Truelab**® analyzer screen.
5. Press start reaction.
6. For **Truelab**® **Uno Dx**, press the eject button to open the chip tray. For **Truelab**® **Duo/Quattro**, the chip tray opens automatically on tapping the “Start Reaction” button.
7. Open a pouch of **Truenat**® **M. leprae** and retrieve the micro PCR chip, microtube and DNase and RNase free pipette tip. Do not open the pouch until ready to test.
8. Place the **Truenat**® **M. leprae** 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 18). 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**® **M. leprae** 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).
10. For **Truelab**® **Uno Dx**, slide the chip tray containing the **Truenat**® **M. leprae** 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**® **M. leprae** micro PCR chip at end of the test and dispose it off as per the section on “Disposal and Destruction” (Section 18).
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.

16. RESULTS AND INTERPRETATIONS

Two amplification curves are displayed on the **Truelab**® analyzer screen 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 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 microbial 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. 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.

17. QUALITY CONTROL PROCEDURES

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

18. DISPOSAL AND DESTRUCTION

1. Submerge the used content such as **Truenat**® **M. leprae** chip, microtube, microtube cap, transfer pipette, 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.

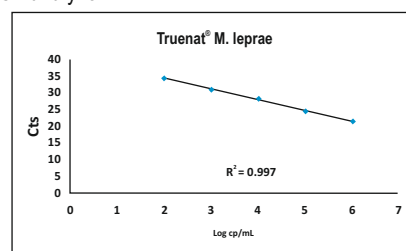
19. SPECIFIC PERFORMANCE CHARACTERISTICS

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**® **M. leprae** assay. Results obtained showed no cross reactivity of the **Truenat**® **M. leprae** assay with the listed organisms.

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

Linearity:

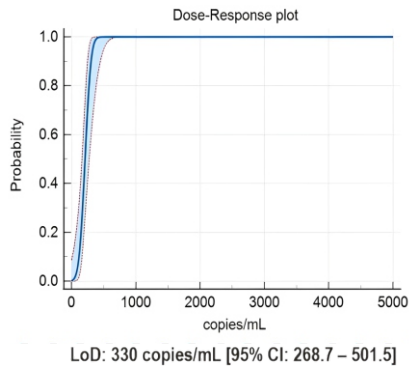
The Linearity analysis was performed according to CLSI Guidelines. Serial dilutions of Quantitative Synthetic *Mycobacterium leprae* DNA BAA-4009SD™ from ATCC ranging from 5.0E+06 to 5.0E+02 copies/mL was made and nucleic acids were extracted on **Trueprep**® **AUTO** Universal Cartridge Based Sample Prep Device in triplicates for each dilution followed by PCR on **Truelab**® Real Time micro PCR analyzer.



The assay is found to be linear over 5 orders of magnitude (from 5.0E+06 to 5.0E+02 copies/mL) for Quantitative Synthetic *Mycobacterium leprae* DNA BAA-4009SD™ from ATCC by **Truenat**® **M. leprae** assay.

Limit of detection(LoD):

The LoD was determined by making dilutions of Quantitative Synthetic *Mycobacterium leprae* DNA BAA-4009SD™ 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**® Real Time micro PCR analyzer. 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 330 copies/mL for Quantitative Synthetic *Mycobacterium leprae* DNA BAA-4009SD™ by **Truenat**® **M. leprae** assay.



data obtained was within the accepted range of $\leq 15\%$ CV for **Truenat[®] M. leprae** assay.

20. REFERENCES

- Alejandra, N. M., Ramanuj, L., Tana, L. P., et al. (2009). Molecular Determination of Mycobacterium leprae Viability by Use of Real-Time PCR. *Journal of Clinical Microbiology*, 47(7), 2124-2130.
- Alejandra, N. M., Constanc F. P. C. B., Jose, A. C. N., et al. (2006). Evaluation of Real-Time and Conventional PCR Targeting Complex 85 Genes for Detection of Mycobacterium leprae DNA in Skin Biopsy Samples from Patients Diagnosed with Leprosy. *Journal of Clinical Microbiology*, 44(9), 3154-3159.
- Jesdawan, W., Somjit, K., Siriphan, S., et al. (1995). Detection of Mycobacterium leprae Infection by PCR. *Journal of Clinical Microbiology*, 33(1), 45-49.
- Mekonnen, K., Assefa, W., Judith, J. R. (1998). Reverse Transcription-PCR Detection of Mycobacterium leprae in Clinical Specimens. *Journal of Clinical Microbiology*, 36(5), 1352-1356.
- Rahul, S., Pushpendra, S., Rajiv, C. M., et al. (2020). Isolation of Mycobacterium lepromatosis and Development of Molecular Diagnostic Assays to Distinguish Mycobacterium leprae and M. lepromatosis. *Clinical Infectious Diseases*, 71(8), 262-269

Robustness:

To determine whether the **Truenat[®] M. leprae** Chip-based Real Time PCR test showed any signs of carryover of PCR products between runs, alternating runs of positive samples and negatives samples were performed. The number of samples tested were 20 positives and 20 negatives. The **Truenat[®] M. leprae** test did not exhibit detectable carryover contamination between positive to negative sample runs.

Reproducibility:

The purpose of this study is to compare the functional performance of the **Truenat[®] M. leprae** assay using three different titres of samples on **Truelab[®]** Real Time 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 (2.01), Inter day (2.56) and Inter Device (1.28) which were in the accepted range of $\leq 15\%$ CV for **Truenat[®] M. leprae** assay.

Interference:

The purpose of this study is to determine the effect of potentially interfering substances on the **Truenat[®] M. leprae** assay. To the samples, different concentrations of interfering substances (endogenous and exogenous) were spiked and then the samples were subjected to extraction on **Trueprep[®] AUTO** Universal Cartridge Based Sample Prep Device. DNA was eluted and PCR was performed on **Truelab[®]** Real Time micro PCR analyzer using **Truenat[®] M. leprae** assays. The presence of the respective potential interference substances did not interfere with the performance of **Truenat[®] M. leprae** assay.

Endogenous Substances	Exogenous Substances
Hemoglobin	EDTA
Albumin	Ciprofloxacin
Triglycerides	Amoxicillin
Bilirubin	Azithromycin
Human DNA	Doxycycline

Precision:

Precision was determined by performing DNA extractions and **Truenat[®] M. leprae** PCR for varying titres of samples over 5 consecutive days. Every day PCR for each titre DNA was run in triplicates. The %CV values obtained for titre 1 (3.90), titre 2 (1.98) and titre 3 (1.36) were within the accepted range of $\leq 15\%$ CV for **Truenat[®] M. leprae** assay.

Clinical validation:

A panel of 30 slit skin samples comprising of 20 negative and 10 positive specimens were tested on three different manufacturing lots of **Truenat[®] M. leprae** assay at Stanley Browne laboratory TLM Community Hospital, Delhi against the reference CE marked kit.

	Reference CE marked kit			
	Positive	Negative	Total	
Truenat[®] M. leprae	Positive	10	0	10
	Negative	0	20	20
	Total	10	20	30
Sensitivity: 100% (95% CI 69.15% to 100%)				
Specificity: 100% (95% CI 83.16% to 100%)				
Accuracy: 100% (95% CI 88.43% to 100%)				

With the consideration of above data, **Truenat[®] M. leprae** assay performed consistently in this study with observed sensitivity of 100% and specificity of 100% in comparison with reference CE marked kit and the inter-lot variation

SYMBOL KEYS

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



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