CE

# Truenat®

## LTS

### Chip-based Real Time PCR Test for Leptospira

## 1. INTENDED USE

**Truenat® LTS (REF 601400005 / 601400020 / 601400025 / 601400050 / 601400100 / 601400200)** is a Chip-based Real Time Polymerase Chain Reaction (PCR) test for the semi-quantitative detection of Leptospira and aids in the diagnosis of Leptospirosis in human blood / serum / plasma, cerebrospinal fluid (CSF) and urine specimen. **Truenat® LTS** runs on **Truelab®** Real Time Quantitative micro PCR Analyzers. **Truenat® LTS** is an *in vitro* diagnostics test meant for professional use only.

## 2. INTRODUCTION

Leptospirosis (also known as Weil's disease) is one of the widespread zoonoses caused by pathogenic spirochetes of the genus *Leptospira* and is found in virtually all tropical and temperate areas of the world. These spirochetes of the genus *Leptospira* are responsible for human and animal leptospirosis, characterised by mild to severe fever to severe multi-organ failure. The incubation time is 5 to 14 days but varies from 75 hrs to a month. Leptospires enter the host via – abrasions, bites, mucus membrane and placenta from urine of infected animals, contaminated food, water or soils. Leptospirosis has a seasonal incidence. Most cases occur during the rainy season in the tropics and late summer or early fall in western countries. Leptospirosis is often misdiagnosed as aseptic meningitis, influenza, dengue, hepatic disease, or pyrexia of unknown origin. Therefore, diagnosis based on laboratory tests rather than on clinical symptoms alone is a must. Dark field microscopy and IgM ELISA are the commonly used laboratory methods but both suffer from poor sensitivity and specificity. Culture is difficult, time consuming and insensitive. Microscopic Agglutination Test (MAT), although considered the gold standard is less sensitive in early phase and is a labour intensive and complicated procedure and is only available in reference laboratories. Molecular tests such as Polymerase Chain Reaction is a sensitive and specific test for detecting the Leptospira DNA in a variety of specimen including blood, CSF and urine. However, molecular tests for Leptospira 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 leptospirosis. This is enabled by making the real time PCR technology rapid, simple, robust and user friendly, thereby 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 Analyzers 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® LTS** 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 infection 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. The **Truenat® LTS** chip also stores information of used test 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® LTS** works on the principle of Real Time Polymerase Chain Reaction (PCR) 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** Cartridge Based Sample Prep Kit. The **Truenat® LTS** 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® LTS** chip and the test is started. A positive amplification causes the labeled fluorescent probes in the **Truenat® LTS** 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, a LTS "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 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 **Truenat® LTS** are repetitive elements which are present across the genome.

## 5. CONTENTS OF THE Truenat® LTS KIT

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

B. Package Insert

| REF | 601400005 | 601400020 | 601400025 | 601400050 | 601400100 | 601400200 |
|---|---|---|---|---|---|---|
| Σ | 5T | 20T | 25T | 50T | 100T | 200T |

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

A. Lysis Buffer (contains lysis cum transport medium)
B. Disposable transfer pipette (graduated)
C. Package Insert

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

## 7. STORAGE AND STABILITY

**Truenat® LTS** is stable for two (2) years from the date of manufacture if stored between 2-30°C. It is also 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** 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 upto 45°C. Do not freeze.

## 8. MATERIALS REQUIRED BUT NOT PROVIDED WITH THE KIT

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

1. **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device (REF603041001 / 603042001).
2. **Truelab® Uno Dx / Truelab® Duo / Truelab® Quattro** Real Time micro PCR Analyzer (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 Sample Pre-treatment Pack (REF60205AB05 / REF60205AB20 / REF60205AB25 / REF60205AB50 / REF60205AB100 / REF60205AB200), **Trueprep® AUTO** Universal Cartridge Based Sample Prep Kit (REF60203AR05 / REF60203AR25 / REF60203AR50 / REF60203AR100) or **Trueprep® AUTO v2** Universal Cartridge Based Sample Prep Kit (REF60207AR05 / REF60207AR25 / REF60207AR50 / REF60207AR100), **Truenat®** Positive Control Kit - Panel IV (REF 801040008), Powder free disposable gloves, waste disposal container with lid.

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

**A. Blood/Serum/Plasma or CSF specimen:**

**Truenat® LTS** requires purified nucleic acids from whole blood / plasma collected

in EDTA anticoagulant or serum 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. Sample must be pre-treated using **Trueprep® AUTO** Universal Sample Pre-treatment Pack. Transfer 250µl of whole blood or 500µl of plasma / serum / CSF specimen using the transfer pipette provided into the Lysis buffer tube provided and mix well.

**B. Urine specimen:**

Collect about 10 ml of first flow of urine (ensuring atleast 2 hours gap from last urination) in a urine collection cup. Transfer 1 ml from the cup to the lysis buffer tube and mix well after tightly closing the cap (Refer to the package insert of **Trueprep® AUTO** Universal Sample Pre-treatment Pack for further details).

**Sample Storage and Transportation:**

Sample pretreatment decontaminates the specimen and makes it ready for storage / transportation / extraction. The specimen in this form is stable for up to three (3) days at 40ºC and one(1) week at 30ºC.

**Nucleic acid extraction:**

Use the entire content of lysis buffer tube 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/AUTO v2** Universal Cartridge Based Sample Prep Kit for details). ⚠ Dispose off the lysis buffer tube with cap 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® LTS** 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.

## 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 "LTS" 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 "LTS" 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® LTS** and retrieve the micro PCR chip, microtube and DNase & RNase free pipette tip.
8. Place the **Truenat® LTS** chip 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.
9. Place the microtube containing freeze dried RT PCR reagents in the microtube stand provided along with the **Truelab®** Real Time micro PCR workstation **after ensuring that white pellet of 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 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® LTS** 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® LTS** 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.
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® LTS** Chip-based Real Time PCR test 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 the **Truelab®** Analyzer manual).
15. Switch off the **Truelab®** Analyzer.

## 15. RESULTS & 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 time taken (Ct) of the specimen will depend on the number of target DNA 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. having a high target load, the IPC may not amplify, however the test run is still considered valid.

## 16. 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®** Positive Control Kit - Panel IV (REF 801040008) 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 content such as **Truenat® LTS** chip, microtube, microtube

cap, pipette tips, lysis buffer tube, urine collection cup 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

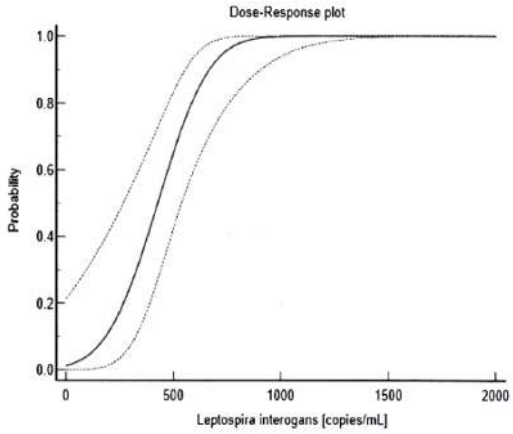
**Analytical Exclusivity (Primer Specificity):**

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® LTS** assay. No cross reactivity in the performance of the **Truenat® LTS** assay was observed with the listed microrganisms.

| | |
|---|---|
| *Candida albicans* | Adenovirus |
| *Chlamydia trachomatis* | Cytomegalovirus |
| *Enterobacter cloacae* | Hepatitis B Virus |
| *Salmonella enterica* | Hepatitis C Virus |
| *Staphylococcus aureus* | HIV Type 1 |
| *Streptococcus mutans* | Epstein-Barr Virus |
| *Escherichia coli* | Herpes Simplex Virus |
| *Gardnerella vaginalis* | Simian Virus |
| *Yersinia enterocolitica* | Human Papillomavirus |
| *Trichomonas vaginalis* | Zika Virus |

**Limit of detection:**

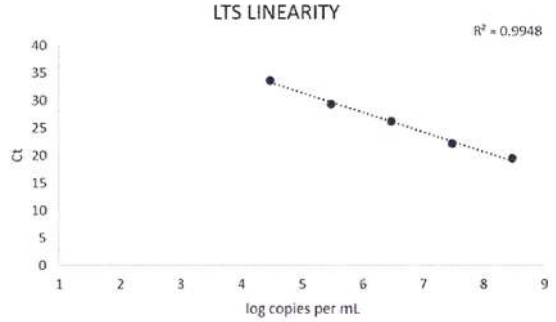
The LoD was determined by making dilutions of *Leptospira interrogans* ATCC 43642™ strain DNA and performing nucleic acid extractions on **Trueprep® AUTO** Sample Prep Device for each of the dilution 24 times followed by PCR on **Truelab®** Analyzer. Probit analysis of the data was used to determine the concentration of the DNA with 95% probability of detection. The LoD was found to be 736.62 copies/ml for *Leptospira interrogans* ATCC 43642™ strain by **Truenat® LTS** assay.




**LoD: 736.62 [95%CI: 611.73 – 1037.76]**

**Linearity:**

The linearity analysis was performed according to CLSI Guidelines. Serial dilutions from 3.01 E+08 to 3.01 E+04 copies/ml of Leptospira interrogans (ATCC® 43642™) culture DNA was made and nucleic acids were extracted on **Trueprep® AUTO** Sample Prep Device in triplicates for each dilution followed by PCR on **Truelab®** Analyzer. The assay is found to be linear over 5 orders of magnitude (from 3.01 E+08 to 3.01 E+04 copies/ml) for LTS DNA from ATCC.




**Robustness:**

Potential sample carryover within the **Truenat® LTS** test was evaluated by testing alternative positive followed by negative samples. The number of samples run was 20 positives and 20 negatives. The results showed no carryover contamination. The **Truenat® LTS** test did not exhibit detectable carry-over contamination from positive to negative samples.

**Reproducibility:**

The purpose of this study is to determine the reproducibility of **Truenat® LTS** assay between three different users, three different devices and five consecutive day study. High, Medium and low titre samples were extracted 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 (0.83), Inter day (1.25) and Inter Device (0.82) which were in the accepted range of ≤15% CV for **Truenat® LTS** assay.

**Precision:**

Precision was tested by performing **Truenat® LTS** assay of High (1.89E+08 copies/ml), Medium (1.89E+06 copies/ml) and Low titre (1.89E+04 copies/ml) DNA for five consecutive days. Every day PCR for each titre DNA was run in duplicates. The %CV values obtained for High titre (4.12), Medium titre (1.53) and low titre (3.52) were within the accepted range of ≤15% CV for **Truenat® LTS** assay.

**Interference:**

**Effect of potentially interfering substances:**

To determine the effect of potentially interfering substances on the performance of **Truenat® LTS** assay. For this study medium load sample were used. To the sample's different concentrations of human DNA (0.4 mg/dl), triglycerides (3.0 mg/dl), bilirubin (20 mg/dl), albumin (9 g/dl) and haemoglobin (500 mg/dl) were spiked and then the samples were extracted with the **Trueprep® AUTO**. DNA was eluted, and PCR was performed on **Truelab® Uno Dx** using **Truenat® L TS** chips.

The presence of human DNA, triglycerides, bilirubin, albumin and haemoglobin did not interfere with the performance of **Truenat® LTS** assay. The CV values obtained were within the accepted range of ≤15%.

**Clinical validation:**

A panel of 30 plasma samples comprising of 20 negative and 10 positive specimens were tested on three different manufacturing lots of **Truenat® LTS** assay at ICMR-Regional Medical Research Centre, Port Blair against ICMR approved Real Time PCR assay. All samples were extracted using **Trueprep® AUTO** Universal Cartridge Based Sample Prep Device and were run in parallel on ICMR approved RT-PCR and **Truelab® Uno Dx** workstation using three manufacturing lots of **Truenat® LTS** assay. All positives (n=10) were detected as positive and all negatives (n=20) also showed negative results on all three lots of **Truenat® LTS** chips confirming 100% concordance with confirmatory test (RT-PCR) used for the study. No significant lot to lot variation was observed.

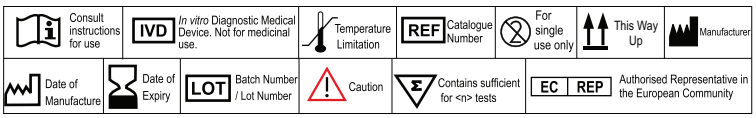
| | | ICMR approved Real-time PCR | | |
|---|---|---|---|---|
| | | Positive | Negative | Total |
| **Truenat® LTS** | Positive | 10 | 0 | 10 |
| | Negative | 0 | 20 | 20 |
| | Total | 10 | 20 | 30 |

With consideration of the above data, **Truenat® LTS** test performed consistently in this study with observed sensitivity of 100% and specificity of 100% in comparison with ICMR approved Real-time PCR and the inter lot variation data obtained was within the accepted range of ≤15% CV for the **Truenat® LTS** test.

## 19. REFERENCES


1. https://www.who.int/topics/leptospirosis/en/
2. https://www.cdc.gov/leptospirosis/index.html
3. Ren, S. X., Fu, G., Jiang, X. G., Zeng, R., Miao, Y. G., Xu, H. & Jiang, H. Q. (2003). Unique physiological and pathogenic features of *Leptospira interrogans* revealed by whole-genome sequencing. Nature, 422(6934), 888-893.
4. Nally, J. E., Whitelegge, J. P., Bassilian, S., Blanco, D. R., & Lovett, M. A. (2007). Characterization of the outer membrane proteome of *Leptospira interrogans* expressed during acute lethal infection. Infection and immunity, 75(2), 766-773.
5. Luchini, D., Meacci, F., Oggioni, M.R., Morabito, G., D'Amato, V., Gabbrielli, M. & Pozzi, G. (2008). Molecular detection of *Leptospira interrogans* in human tissues and environment samples in a lethal case of *leptospirosis. International Journal of legal medicine,* 122 (3), 229.

**SYMBOL KEYS**

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