**1. INTENDED USE**

Truenat™ Dengue (REF 601050005 / 601050020) is a chip-based Real Time Reverse Transcription Polymerase Chain Reaction (RT PCR) test for the quantitative detection and identification of Dengue virus in human serum, plasma, whole blood, and EDTA plasma. This test is also used to identify Dengue virus in the semen of men and in the peripheral blood mononuclear cells (PBMCs) of patients with Dengue. The test runs on the TrueLab™ Uno and TrueLab™ Uno Dx Real time micro PCR Analyzer.

**2. INTRODUCTION**

Dengue infection is a leading cause of morbidity and mortality in the tropical and subtropical regions of the world with a huge economic cost associated with it. As many as 100 million people are estimated to be infected with Dengue yearly. Dengue infection is caused by any one of the four related serotypes of the Dengue virus (DEN 1 to DEN 4). Dengue virus, once transmitted by the bite of an infected, day biting, Aedes mosquito (Aedes aegypti and Aedes albopictus) is characterized by a sudden onset of high fever, headache, retro orbital pain in the back and limbs (break-bone fever), lymphadenopathy and maculopapular rash. Depending on the immune response, Dengue infection is classified as either primary or secondary. Primary Dengue infection is usually self limiting but can cause Life threatening conditions such as Dengue Haemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS) are more severe forms of Dengue usually associated with secondary infection and is fatal if not treated early. Thus early diagnosis and treatment is crucial in the management of Dengue. Current methods of diagnosis of the disease include viral isolation, serologic tests and molecular methods. Serology based tests qualitatively detect IgM and IgG antibodies that appear much later during the convalescent stage or detect the NS1 antigen during the late acute phase and early convalescent phase.

However, these methods are known to show cross reactivity with other flaviviruses and have limitations of sensitivity. Viral isolation and molecular techniques such as Polymerase chain reaction (PCR) or Real Time PCR are much more sensitive and confirm infection with Dengue, immediately upon onset of symptoms. In real-time reverse transcription PCR (RT RCP) the virus amplification curve is shown on a graph (v=RNA concentration over time), the Ct value is linearly correlated with amount of target RNA (proportional to the amount of target nucleic acid in the sample). Ct value is inversely proportional to the amount of target nucleic acid in the sample (i.e. the lower the Ct level the greater is 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. Ct value 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. Ct value is inversely proportional to the amount of target nucleic acid in the sample.

**3. PRINCIPLE OF THE TEST**

Truenat™ Dengue works on the principle of Real Time Reverse Transcription Polymerase Chain Reaction (RT PCR). The RNA from the patient blood/serum/plasma sample is first extracted using Universal Cartridge Based Sample Prep Device and Trueprep™ MAG Blood Sample Prep Kit or using Truenat™ AUTO Universal Card based Sample Prep Device and Trueprep™ AUTO Universal Card based Sample Prep. The Truenat™ Dengue chip is placed on the chip tray of the TrueLab™ Real time micro PCR Analyzer.

**4. TARGET SELECTION**

The target sequence for this kit has been taken from the 3’ Untranslated region (UTR) of the Dengue genome. The region selected is specific to and represents all four serotypes (DEN 1 to DEN 4) of Dengue virus.

**5. CONTENTS OF THE KIT**

**A. Test kit:**

- Individual sealed pouches, each containing a
  1. Truenat™ Dengue Chip
  2. Microtube with freeze dried IP PCR reagents
  3. DNase & RNase free pipette tip
  4. Desiccant pack

**B. Package insert:**

- Lysis buffer
- Disposable transfer pipette (graduated).

**6. CONTENTS OF THE Trueprep™ AUTO Universal Sample Pre-treatment Kit (only for Trueprep™ AUTO users)**

- Lysis buffer
- Disposable transfer pipette (graded).

**7. STORAGE AND STABILITY**

Truenat™ Dengue chip is stable for two (2) years from the date of manufacture if stored between 2-30°C. It is also stable for up to six (6) months at temperatures up to 40°C and one (1) month at elevated temperatures up to 45°C. Avoid exposure to light or elevated temperatures (above recommended levels). Do not freeze.

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

- Trueprep™ AUTO Realtime micro PCR Workstation (REF 62301001) consisting of
  1. Trueprep™ MAG / AUTO Sample Prep Device (REF 603040001/603041001/603042001)
  2. Truelab™ Uno / TrueLab™ Uno Dx Real time micro PCR Analyzer (REF 603020001/6030201001)
  3. Trueprep™ micro PCR Printer (REF 603050001)
  4. TruSample™ SP fixed volume precision L pipet - 6 µl (REF 60460006)
  5. Trueprep™ Microtube Stand (REF 603050001/6030501001)
  6. Trueprep™ AUTO Universal Cartridge Based Sample Prep Kit (REF 6020301005/6020301006/6020301025), Trueprep™ Universal Control Kit (REF 601100008), DNase and RNase-free pipette tips with filter barrier, and may also be procured from Molbio, Powder free disposable gloves, waste disposal container with lid.

**9. SPECIMEN PREPARATION FOR EXTRACTION WITH Trueprep™ MAG**

**A. Disposable transfer pipette (graduated).**

- Universal Cartridge based Sample Prep Device and Trueprep™ AUTO Universal Cartridge based Sample Prep Device.

**B. Disposable transfer pipette (graduated).**

- Universal Cartridge based Sample Prep Device and Trueprep™ AUTO Universal Cartridge based Sample Prep Kit.

**10. SPECIMEN PREPARATION FOR EXTRACTION WITH Trueprep™ AUTO**

- Reagents and specimen are extracted using the Trueprep™ AUTO Universal Cartridge based Sample Prep Device and Trueprep™ AUTO Universal Cartridge based Sample Prep Kit.

**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 Trueprep™ 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 package 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™ Dengue 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 are used.

**13. PROCEDURAL LIMITATIONS**

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

1. Though very rare, mutations within the highly conserved regions of the target genome where the assay primers bind can result in false negativity.
2. Viral isolation and molecular techniques such as Polymerase Chain reaction (PCR) or Real Time PCR requires purified nucleic acids from whole blood/plasma collected in EDTA anticoagulant or serum that are extracted using the Trueprep™ AUTO Sample Prep Device and Trueprep™ AUTO Blood Sample Prep Kit.

**14. NON DENDRAL**

- Trueprep™ Dengue requires purified nucleic acids from whole blood/plasma collected in EDTA anticoagulant or serum that are extracted using the Trueprep™ AUTO Universal Cartridge based Sample Prep Device and Trueprep™ AUTO Universal Cartridge based Sample Prep Kit.

**15. CRITERIA FOR INTERPRETATION**

- Trueprep™ MAG Blood Sample Prep Kit (REF 603020001/6030201001).

**16. ECONOMIC BENEFITS**

- Trueprep™ AUTO Universal Cartridge based Sample Prep Kit (REF 6020301005/6020301006/6020301025), Trueprep™ Universal Control Kit (REF 601100008), DNase and RNase-free pipette tips with filter barrier and may also be procured from Molbio, Powder free disposable gloves, waste disposal container with lid.

**17. DATA DISCLOSURE**

- Trueprep™ AUTO Universal Cartridge based Sample Prep Device and the package insert of Trueprep™ AUTO Blood Sample Prep Kit for details.

**18. SPECIMEN STORAGE AND TRANSPORTATION**

- True prep Test Reportguide (for use with Trueprep™ AUTO Universal Cartridge based Sample Prep Device and the package insert of Trueprep™ AUTO Universal Cartridge based Sample Prep Kit) for details.

**19. SAFETY INSTRUCTIONS**

- Trueprep™ AUTO Universal Cartridge based Sample Prep Device and the package insert of Trueprep™ AUTO Universal Cartridge based Sample Prep Kit for details.

- Trueprep™ AUTO Universal Cartridge based Sample Prep Device and the package insert of Trueprep™ AUTO Universal Cartridge based Sample Prep Kit.
To determine the range of the assay, isolated RNA from standard cultures was used as source RNA. Standard curves were developed using serial dilutions of the source RNA in sterile TE buffer. The various dilutions were subjected to reverse transcription and the resulting cDNAs were tested using Truenat™ Dengue chip-based Real Time PCR test. The Ct values obtained were found not to be significantly impacted (Ct Average = 33.46; Standard Deviation = 0.31, Variance = 0.10; Co-efficient of variation = 1%) by the presence of elevated biochemical parameters as described above.

Effect of elevated biochemical blood parameters:

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<th>Sr. No.</th>
<th>Sample I.D.</th>
<th>Cholesterol</th>
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<th>Triglycerides</th>
<th>LDL</th>
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<td>38</td>
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DISPLOCATION AND DESTRUCTION

1. Submerge the used Truenat™ Dengue 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 contaminated fluid or water).
4. Do not autoclave or store solutions containing sodium hypochlorite.
5. Chemicals should be handled in accordance with Good Laboratory Practice and disposed off according to the local regulations.

14. CLEANING AND DECONTAMINATION

1. Spills of potentially infectious material should be cleaned up immediately with absorbent paper and the contaminated area should be disinfected 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 found to be linear over 5 orders of magnitude and transcription and the resulting cDNAs were tested on clinical specimen.

15. TEST PROCEDURE

(Also please refer the Truelab™ Uno Truelab™ Uno Dx 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 proceed to step 3.
3. Select user and enter password.
4. Select the test profile for “DENGUE” on the Analyzer screen.
5. Enter the patient details as prompted in the Truelab™ Analyzer screen.
6. Press Start button.
7. The press the eject button to open the chip tray.
8. Open a pouch of Truenat™ Dengue 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™ Dengue 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.
11. 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 RT PCR reagents remains at the bottom of the microtube. Remove the microtube cap and dispose it 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 RNA from the ELute Collecte 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 center white reaction well of the Truenat™ Dengue 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. Slide the chip tray containing the Truenat™ Dengue chip-based Real Time PCR test loaded with the sample, into the Truelab™ Analyzer.
13. Press the power button on the Analyzer to turn it on. The green LED should glow.
15. Read the result from the screen.
16. Take out the Truenat™ Dengue micro PCR chip at the end of the test and dispose it as per the section on “Disposal and Destruction” (Section 18).
17. 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 at any time later. (Refer to Truelab™ Analyzer manual).
18. Switch off the Truelab™ Analyzer.

16. RESULTS & INTERPRETATIONS

Two amplification curves are displayed on the Truelab™ Real Time micro PCR 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 Ct values obtained will depend on the number of target RNA 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 will also display the Ct value and the Viruses per μl (Viruses/ml) for positive specimen. The result screen also displays the validity of the test run as “VALID” or “INVALID”. Invalid samples must be repeated with fresh specimen from the sample preparation stage. While IPC will co-run, the results screen will display “DETECTED” for Positive result or “NOT DETECTED” for Negative result.

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™ Dengue 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 contaminated fluid or water).
4. Do not autoclave or store 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

To determine the range of the assay, isolated RNA from standard cultures was used as source RNA. Standard curves were developed using serial dilutions of the source RNA in sterile TE buffer. The various dilutions were subjected to reverse transcription and the resulting cDNAs were tested using Truenat™ Dengue chip-based Real Time PCR test. The Ct values obtained were plotted against the RNA concentration. The assay was found to be linear over 5 orders of magnitude and could detect as low as 10 copies of RNA per reaction.