CE

Truenat®

Dengue

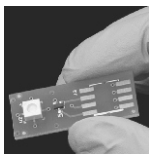
Chip-based Real Time PCR Test for Dengue

1. INTENDED USE

REF **Truenat® Dengue (REF 601050005 / 601050020 / 601050025 / 601050050 / 601050100 / 601050200)** is an automated point-of-care or near patient Chip-based Real Time Reverse Transcription Polymerase Chain Reaction (RT-PCR) test for the quantitative detection of Dengue virus in human blood/serum/plasma specimen and aids in the diagnosis of infection with Dengue. **Truenat® Dengue** runs on the **Truelab®** Real Time Quantitative micro PCR Analyzer. **Truenat® Dengue** 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

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, DEN-2, DEN-3, or DEN-4). Dengue, an RNA virus, is transmitted by the bite of an infected, day biting, *Aedes* mosquito (*Aedes aegypti* and *Aedes albopictus*) and 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 primary or secondary. Primary dengue infection is usually self limiting and uncomplicated. 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 fever. 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, during the acute (viremic) phase. However, viral isolation and 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 **Truenat®** point-of-care real time PCR System enables decentralization and near patient diagnosis of dengue by making 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 Analyzer and **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device and room temperature stable **Truenat®** Chip-based Real Time PCR test and **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit 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® Dengue is a disposable, room temperature stable, Chip-based Real Time PCR test with dried $MgCl_2$ in reaction well and freeze dried RT-PCR reagents in microtube for performing a Real Time PCR test for detection of dengue and runs on the **Truelab®** Real Time Quantitative micro PCR Analyzers.All the components of **Truenat®** pouch are nuclease-free. It requires only six (6) µL of purified RNA 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® Dengue** chip also stores information of used chip 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® Dengue works on the principle of Real Time Reverse Transcription Polymerase Chain Reaction (RT-PCR) based on Taqman chemistry. The patient sample (blood / serum / plasma) is first pre-treated using the **Trueprep® AUTO** Universal Sample Pre-treatment Pack. The RNA 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), it is a synthetic plasmid construct provided in a stable formulation preloaded into Cartridges which is co-extracted along with sample nucleic acids, thereby validating the process from extraction to PCR run. The RNA extract is analyzed using the **Truenat® Dengue** Chip-based Real Time Reverse Transcription Polymerase Chain Reaction (RT-PCR) test and the **Truelab®** Real Time Quantitative micro PCR Analyzer. The **Truenat® Dengue** chip is placed on the chip tray of the **Truelab®** Real Time Quantitative micro PCR Analyzer. Six (6) µL of the purified RNA is then dispensed using the provided calibrated micropipette and tip into the microtube containing freeze dried RT-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® Dengue** chip and the test is inserted in the Truelab® Real Time Quantitative micro PCR Analyzer where the RNA is first converted into complementary DNA (cDNA) by the RT enzyme and further thermal cycling takes place. A positive amplification causes the dual labeled fluorescent probe in the **Truenat® Dengue** chip to release the fluorophores in an exponential manner and the emitted light 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 Dengue"DETECTED" or "NOT DETECTED" result is displayed and in positive cases, Ct values and Copies/ml 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 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 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 Truenat® Dengue KIT

A. Individually sealed pouches
B. Package Insert
   Each individually sealed pouch contains:
   1. **Truenat® Dengue** micro PCR chip (1 Nos.)
   2. Microtube with freeze dried RT-PCR reagents (1 Nos.)
   3. DNase and RNase free pipette tip (1 Nos.)
   4. Desiccant pouch (1 Nos.)

REF	601050005	601050020	601050025	601050050	601050100	601050200
Σ	5T	20T	25T	50T	100T	200T

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

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

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

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

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

8. STORAGE, HANDLING AND STABILITY

1. **Truenat® Dengue** 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.
2. **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.
3. **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**.**
4. 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.
5. Do not use the pouch if torn.
6. 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,

1. **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device (REF 603041001 / 603042001).
2. **Truelab® Uno Dx / Truelab® Duo / Truelab® Quattro** Real Time micro PCR Analyzer (REF 603021001/603022001/603023001).
3. **Truelab®** micro PCR Printer (REF 603050001).
4. **Truepet®** SPA fixed volume precision micropipette - 6 µl (REF 604070006).
5. **Truelab®** Microtube Stand (REF 603070001).
6. 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)**, Truenat®** Positive Control Kit - Panel II (REF 801020008), Powder free disposable gloves, waste disposal container with lid and sodium hypochlorite.

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

Truenat® Dengue 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 specimen using the transfer pipette provided into the lysis buffer tube provided and mix well (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. The specimen in this form is stable for up to 3 days at 40ºC and 1 week at 30ºC.

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/AUTO v2 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

IVD 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 **Truenat®** point-of-care real time PCR system before use.
5. Good laboratory practices are recommended to avoid contamination of specimens or reagents.
6. All materials of human origin should be handled as though potentially infectious.
7. Do not pipette any material by mouth.
8. Do not eat, drink, smoke, apply cosmetics or handle contact lenses in the area where testing is done.
9. Use protective clothing and wear disposable gloves when handling samples and while performing sample extraction.
10. Do not substitute assay components / reagents with any other components / reagents.
11. Each single-use **Truenat®** chip is used to process one test. Do not reuse chip.

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® Dengue** 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 run are used.
4. 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

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 Quantitative 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 "Dengue" to be run from the Profiles Screen on the analyzer screen. For **Truelab**® **Duo/Quattro**, select the Bay (I/II) for **Duo** and (I/II/III/IV) for **Quattro** from the Status Screen to view the Profiles Screen. Select the test profile for "Dengue" to be run from the Profiles Screen, on the analyzer screen.
4. Enter the patient details as prompted in the **Truelab**® Analyzer screen.
5. Press Start Test.
6. For **Truelab**® **Uno Dx,** Press the eject button to open the chip tray. For **Truelab**® **Duo/Quattro**, the chip tray opens automatically on tapping the "Start Test" button.
7. Open a pouch of **Truenat**® **Dengue** 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**® **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 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 RT-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 RNA 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**® **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).
10. For **Truelab**® **Uno Dx**, slide the chip tray containing the **Truenat**® **Dengue** 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**® **Dengue** Chip-based Real Time PCR test 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 the **Truelab**® Analyzer manual).
15. Switch off the **Truelab**® Analyzer.

16. RESULTS AND 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 Cycle threshold (Ct) will depend on the number of target nucleic acids in the sample. The target curve will remain horizontal throughout the test duration and the IPC curve will take an exponential path in case of negative samples. In case the IPC curve remains horizontal in a negative sample, the test is considered as Invalid. At the end of the test run, the results screen will display "DETECTED" for Positive result or "NOT DETECTED" for Negative result. The result screen would also display the Ct value and the copies per ml (cp/ml) 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 positive and negative controls from time to time. The **Truenat**® Positive Control Kit - Panel II (REF 801020008), 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

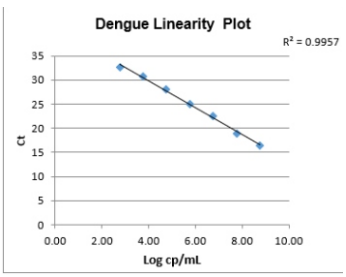
1. Submerge the used **Truenat**® **Dengue** chip, microtube, microtube cap, transfer pipette, pipette tips, lysis buffer tube etc. in freshly prepared 0.5 % sodium hypochlorite solution for 30 minutes before disposal as per the standard medical waste disposal guidelines.
2. Disinfect the solutions and/or 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 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

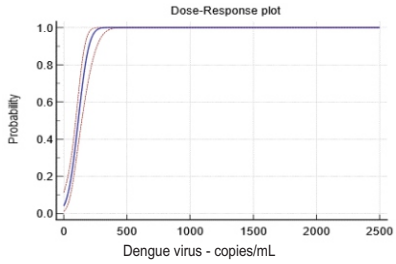
Analytical Exclusivity (Primer specificity): The following microorganisms were evaluated *in silico* from the NCBI database using the NCBI nucleotide blast and primer blast tools to determine for potential cross-reactivity in the **Truenat**® **Dengue** assay. Results obtained showed no cross-reactivity of the **Truenat**® **Dengue** test with the listed organisms.

Organisms	Organisms
Acinetobacter anitratus	Human papilloma virus
Candida albicans	Adenovirus
Enterococcus faecalis	Cytomegalovirus
Salmonella enterica	Hepatitis B virus
Staphylococcus aureus	Hepatitis C virus
Streptococcus mutans	Human Immunodeficiency virus
Escherichia coli	Epstein-Barr virus
Gardnerella vaginalis	Herpes Simplex virus
Neisseria gonorrhoeae	Simian virus
Trichomonas vaginalis	
Enterobacter cloacae	

Linearity: The linearity analysis was performed according to CLSI Guidelines. Serial dilutions of the Dengue RNA cloned in a plasmid was made from 5.73E+08 copies/mL to 5.73E+02 copies/mL and nucleic acids were extracted on **Trueprep**® **AUTO** Universal Cartridge Based Sample Prep Device in triplicates for each dilution followed by PCR on **Truelab**® analyzer. The assay is found to be linear over 7 orders of magnitude (from 5.73E+08 to 5.73E+02 copies/mL) for Dengue RNA by **Truenat**® **Dengue** test.



Limit of detection (LoD): The LoD was determined by making dilutions of Zeptomertix culture of Dengue and performing nucleic acid extractions on **Trueprep**® **AUTO** Universal Cartridge Based 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 RNA with 95% probability of detection. The LoD was found to be 222.99 copies/mL for the Dengue Zeptomertix culture by **Truenat**® **Dengue** test.



LoD: 222.99 copies/mL [95% CI: 183.93- 298.06]

Dose (cp/mL)	Total sample runs	Percentage Positivity
2290	24	100
1145	24	100
572.5	24	100
286.25	24	95.8
143.12	24	79.1
71.56	24	29.1
35.8	24	8.33
0	24	0

Robustness: To determine whether the **Truenat® Dengue** test showed any signs of carryover of PCR products between runs, potential sample carryover within the **Truenat® Dengue** test was evaluated by testing alternate positive followed by negative samples. The number of samples run were 20 positives and 20 negatives. The **Truenat® Dengue** test did not exhibit detectable carryover contamination from positive to negative samples.

Reproducibility: The purpose of this study is to compare the functional performance of the **Truenat® Dengue** 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 (2.96), inter day (1.54) and inter device (1.22) which were in the accepted range of ≤15% CV for **Truenat® Dengue** assay.

Interference: To determine the effect of interfering substances on the **Truenat® Dengue** assay one negative and one positive sample was used. To the sample different concentrations of interfering substances were spiked and then the samples were subjected to sample prep on **Trueprep® AUTO**. The RNA was eluted and PCR was performed on **Truelab®** devices using **Truenat® Dengue** tests.

Endogenous analytes	
Interfering substance	**Concentration**
Control	NA
Human DNA	0.4 mg/dL
Hemoglobin	500 mg/dL
Bilirubin	20 mg/dL
Triglycerides	3 mg/dL
Serum albumin	9 g/dL

Exogenous analytes	
Interfering substance	**Concentration**
EDTA	100 µg/mL
Ibuprofen	1 mg/mL
Creatinine	1 mg/mL
Chloroquine	1 mg/mL
Salicylic Acid	100 µg/mL
Acetaminophen	1 mg/mL
Tylenol	100 µg/mL
Dipyrone	1 mg/mL

The presence of the above tested potential interference substances did not interfere with the performance of **Truenat® Dengue** test.

Precision: Precision was tested by performing **Truenat® Dengue** assay of high, medium and low titre RNA for five consecutive days. Every day PCR for each titre RNA was run in duplicates. The %CV values obtained for high (4.06), medium (2.33) and low (2.49) were within the accepted range of ≤15% CV for **Truenat® Dengue** assay.

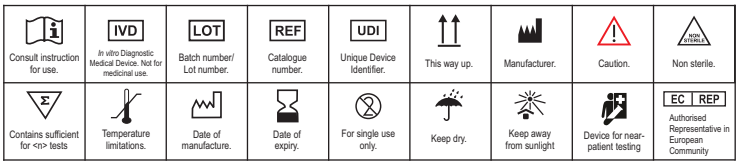
20 REFERENCES

1. http://www.cdc.gov/dengue/.
2. http://www.who.int/topics/dengue/en/.
3. Vaughn DW, et al. Dengue viremia titer, antibody response pattern and virus serotype correlate with disease severity. J. Infect. Dis. 181: 2-9.
4. SAM Kularatne et al. (2009). Concurrent outbreaks of Chikungunya and Dengue fever in Kandy, Sri Lanka, 2006-07: A comparative analysis of clinical and laboratory features. Postgrad Med J 2009; 85:342-346.
5. T. Mirawati Sudiro et. al. (1997) Rapid diagnosis of dengue viremia by Reverse-transcriptase Polymerase Chain Reaction using 3'-Noncoding region universal primers. Am. J. Trop. Med. Hyg., 56(4), 1997, pp. 424 - 429.

21. REVISION HISTORY

Section	Description of changes
Throughout	Symbols are added as per regulatory requirements
19	Specific Performance characteristics is updated
21	Added Revision History table

SYMBOL KEYS



Molbio Diagnostics Private Limited

Registered Office and Manufacturing Unit - I:
Plot No. L-46, Phase II D, Verna Industrial Estate, Verna, Goa - 403 722, INDIA
Manufacturing Unit - II:
Plot No. L-42, Phase II B, Verna Industrial Estate, Verna, Goa - 403 722, INDIA
www.molbiodiagnostics.com
Email: sales@molbiodiagnostics.com (Sales Enquiries)
customersupport@molbiodiagnostics.com (Feedback and Customer Support)

EC REP Qarad EC-REP BV, Pas 257, 2440 Geel, Belgium

TNDEN/0724/V-04
English - July 2024