CE

Truenat®

Dengue/Chikungunya

Chip-based Real Time Duplex PCR Test for Dengue and Chikungunya

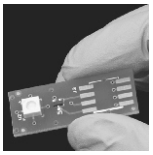
1. INTENDED USE

Truenat® Dengue/Chikungunya (REF 601040005 / 601040020 / 601040025 / 601040050 / 601040100 / 601040200) is a chip-based Real Time Reverse Transcription Polymerase Chain Reaction (RT PCR) test for the quantitative detection of **Dengue and Chikungunya** virus in human blood/serum/plasma specimen and aids in the differential diagnosis of infection with **Dengue** and/or **Chikungunya** virus. **Truenat® Dengue/Chikungunya** runs on the **Truelab®** Real Time Quantitative micro PCR Analyzers. **Truenat® Dengue/Chikungunya** is an *in vitro* diagnostics test meant for professional use only.

2. INTRODUCTION

Dengue and Chikungunya are both acute febrile diseases caused by different viruses. Both are RNA viruses and are both spread by Aedes mosquito (*Aedes aegypti* and *Aedes albopictus*). Chikungunya is often mis-diagnosed as Dengue because of common symptoms, common vectors and common transmission conditions. Common symptoms include high fever, headache, eye pain, joint pain, rashes and lethargy. Early detection and differential diagnosis is critical as Dengue is much more dangerous and may need emergency medical intervention. Also co-infection with the two viruses is possible.

Dengue infection can have serious consequences if not diagnosed and treated early. 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). 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 are fatal if not treated early. Though Chikungunya is not associated with severe, life threatening complications, children, elderly and sick people are at higher risk of developing severe disease. The risk of death is around 1 in 1000, with rare instances of neurological disorders reported. The disease is characterized by intense, debilitating joint pains that can last for days to years.



Current methods of diagnosis of the two diseases include viral isolation, serologic and antigen detection tests and molecular methods. Serology based tests qualitatively detect IgM and IgG antibodies that appear later during the convalescent stage of infection and can be used as follow up tests. Antigen tests, especially for Dengue, can pick up infection earlier. However, these methods are known to show cross reactivity and also 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 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 **Truelab®** Real Time micro PCR System enables decentralization and near patient detection, differential diagnosis and viral load monitoring of Dengue/Chikungunya by making real time PCR technology rapid, simple, robust and user friendly and offering "sample to result" capability even at resource limited settings. This is achieved through a combination of lightweight, portable, mains / battery operated **Truelab®** Real Time 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 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/Chikungunya is a disposable, room temperature stable, micro PCR test with dried $MgCl_2$ in reaction well and freeze dried RT PCR reagents in microtube for performing Real Time RT-PCR test for Dengue/Chikungunya virus and runs on the **Truelab®** Real Time Quantitative micro PCR Analyzer. 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. The **Truenat® Dengue / Chikungunya** chip also stores information of used chips to prevent any accidental re-use of the chip.

3. PRINCIPLE OF THE TEST

Truenat® Dengue/Chikungunya works on the principle of Real Time Reverse Transcription Polymerase Chain Reaction (RT PCR) based on Taqman chemistry. The RNA from the patient sample is first extracted using **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device and **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit. The **Truenat® Dengue/Chikungunya** chip is placed on the chip tray of the **Truelab®** Real Time micro PCR Analyzer. Six (6) µL of the purified RNA is then dispensed using the provided 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/Chikungunya** 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 labeled fluorescent probes in the **Truenat® Dengue/Chikungunya** 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, Dengue/Chikungunya "DETECTED" or "NOT DETECTED" result is displayed and in positive cases, Ct values and copies per milliliter (copies/mL) 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 20,000 results in **Truelab® Uno Dx / Truelab® Duo / Truelab® Quattro** can be stored on the analyzer for future recall and reference.

4. TARGET SELECTION

Dengue: 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.

Chikungunya: The target sequence for this kit has been taken from the Non-structural protein *4(nsP4)* gene of the Chikungunya genome. The region selected is specific for Chikungunya virus.

5. CONTENTS OF THE Truenat® Dengue/Chikungunya KIT

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

B. Package Insert.

REF	601040005	601040020	601040025	601040050	601040100	601040200
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. STORAGE AND STABILITY

Truenat® Dengue/Chikungunya chip 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 (REF 603020001/603021001/603022001/603023001).
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 II (REF 801020008), Powder free disposable gloves, waste disposal container with lid.

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

Truenat® Dengue/Chikungunya 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 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 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.

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® Dengue/Chikungunya** 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.

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 "Dengue/Chikungunya" 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 "Dengue/Chikungunya" 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® Dengue/Chikungunya** and retrieve the chip-based Real Time PCR test and the microtube.
8. Place the **Truenat® Dengue/Chikungunya** 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 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 RNA 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® Dengue/Chikungunya** 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® Dengue/Chikungunya** 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® Dengue/Chikungunya** 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

Three amplification curves are displayed on the **Truelab®** Real Time Quantitative 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 time taken (Ct) of the specimen will depend on the number of target RNA 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 display the Ct value and the copies per milliliter (copies/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.

16. QUALITY CONTROL PROCEDURES

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

17. DISPOSAL AND DESTRUCTION

1. Submerge the used **Truenat® Dengue/Chikungunya** 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.

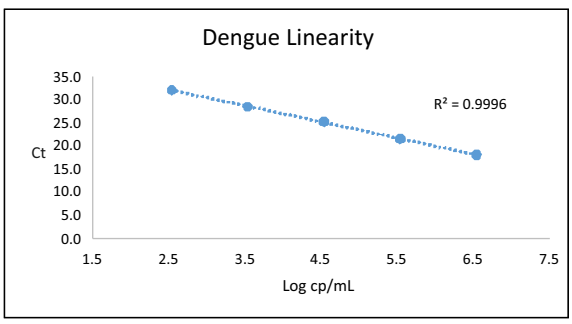
18. SPECIFIC PERFORMANCE CHARACTERISTICS

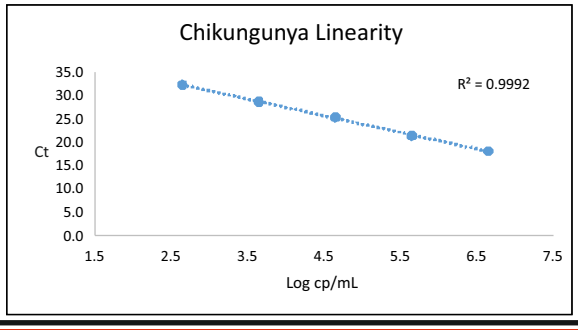
Analytical Exclusitivity (Primer specificity): The following viruses and microorganisms were evaluated *in silico* for potential cross-reactivity in the **Truenat® Dengue/Chikungunya** assay. No interference in the performance of the **Truenat® Dengue/Chikungunya** assay was observed.

Bacteria	Virus
Acinetobacter anitratus	Human papilloma virus
Candida albicans	Adenovirus
Enterobacter cloaceae	Cytomagalovirus
Salmonella enterica	Hepatitis B virus
Staphylococcus aureus	Hepatitis C virus
Streptococcus mutans	Human Immunodeficiency virus
Escherichia coli	Epstein-Barr virus
Gardenerella vaginalis	Herpes Simplex virus
Neisseria gonorrheae	Simian virus
Trichomonas vaginalis	
Enterococcus faecalis	

Linearity and Assay range:

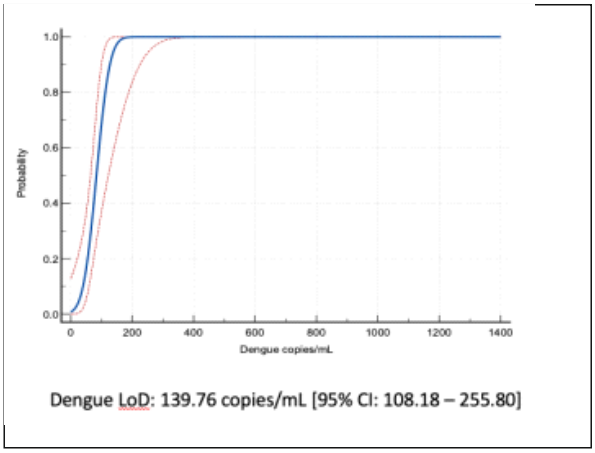
The Linearity analysis was performed according to CLSI Guidelines. Serial dilutions of RNA from 1.0E+06 to 1.0E+02 copies/mL were made and nucleic acids were extracted on **Trueprep® AUTO** Sample Prep Device in triplicates for each dilution followed by PCR on **Truelab®** Real Time micro PCR Analyzer using **Truenat® Dengue/Chikungunya** test. The assay is found to be linear over 5 orders of magnitude (from 1.0E+06 to 1.0E+02 copies/mL) for Dengue & Chikungunya culture from Zeptometrix as depicted in the given graph.

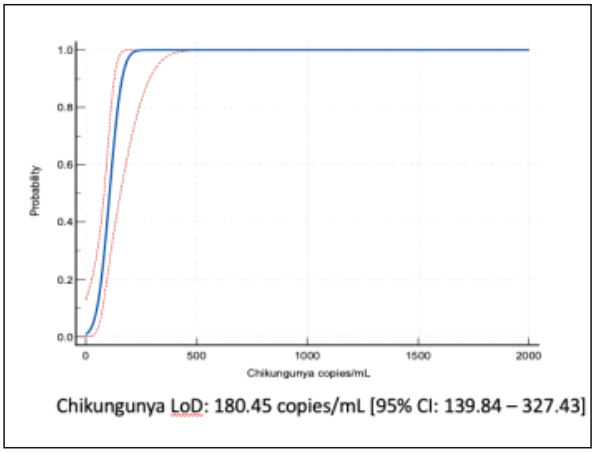




Limit of detection:

The LoD was determined by making dilutions of Zeptomertix culture of Dengue & Chikungunya sample and performing nucleic acid extractions on **Trueprep® AUTO** Sample Prep Device for each of the dilution 10 times followed by PCR on **Truelab®** Real Time micro PCR analyzers. Probit analysis of the data was used to determine the concentration of the respective RNA with 95% probability of detection. The LoD was found to be 139.76 copies/mL for Dengue and 180.45 copies/mL for Chikungunya by **Truenat® Dengue/Chikungunya** assay for Zeptometrix culture.





Robustness:

To determine whether the **Truenat® Dengue/Chikungunya** 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. 10 positive and 10 negative samples were used for the study. The **Truenat® Dengue/Chikungunya** 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® Dengue/Chikungunya** assay using three different titres of IVT RNA samples on **Truelab® Uno Dx** 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 Dengue as Inter User (2.65), Inter day (1.64) and Inter Device (1.87) and for Chikungunya as Inter User (1.35), Inter day (0.92) and Inter Device (1.59) which were in the accepted range of ≤15% CV for **Truenat® Dengue/Chikungunya** assay.

Precision of Truenat® Dengue/Chikungunya assay:

Precision was tested by performing **Truenat® Dengue/Chikungunya** assay on High (1.0E+05 cells/mL), Medium (1.0E+04 cells/mL) and Low (1.0E+03 cells/mL) titres of RNA for Dengue while High (1.80E+07 copies/mL), Medium (1.80E+05 copies/mL) and Low (1.80E+03 copies/mL) titres of IVT RNA for Chikungunya for five consecutive days. Every day PCR for each titre RNA was run in triplicates for Dengue and in duplicates for Chikunguya. The %CV values obtained for High titre (3.29), Medium titre (2.6) and low titre (3.36) for Dengue while High titre (2.16), Medium titre (1.86) and low titre (2.50) for Chikungunya were within the accepted range of ≤15% CV for **Truenat® Dengue/Chikungunya** assay.

19. REFERENCES

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Chikungunya virus from *Aedes aegypti* mosquitoes collected in the town of Yawat, Pune. District, Maharashtra State, India. Acta. Virol., 2001; 45 (5-6): 305-309.
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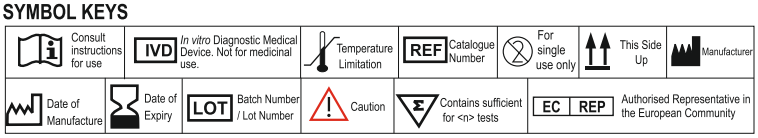
SYMBOL KEYS



molbio®

Molbio Diagnostics Private Limited

Registered Office & 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

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