

TruenatTM

Chikungunya

Chip-based Real Time PCR Test for Chikungunya

1. INTENDED USE

TruenatTM Chikungunya (REF 601060005/601060020/601060050) is a chip-based Real Time Reverse Transcription Polymerase Chain Reaction (RT PCR) test for the quantitative detection and diagnosis of Chikungunya virus in human blood/serum/plasma specimen and aids in the diagnosis of infection with Chikungunya. **TruenatTM Chikungunya** runs on the **TruelabTM** Real Time Quantitative micro PCR Analyzers.

2. INTRODUCTION

Chikungunya, an RNA virus, is transmitted by the bite of an infected, day biting, Aedes mosquito (*Aedes aegypti* and *Aedes albopictus*). Infection with Chikungunya is characterized by a sudden onset of high fever and symptoms similar to influenza mostly accompanied by intense, debilitating joint pains in the extremities (ankle, wrists and/or fingers). Other possible symptoms are swollen hands and/or feet, skin rashes and light hemorrhages. Usually the course of the disease is benign and the disease heals spontaneously in few days to few weeks. Chikungunya infections often gets mis-diagnosed as Dengue because of common symptoms, common vector and common transmission conditions. Current methods of diagnosis of the disease include viral isolation, serologic tests and molecular methods. Serology based tests qualitatively detect antibodies that appear later during the infection. 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 with Chikungunya, immediately upon onset of symptoms. 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.

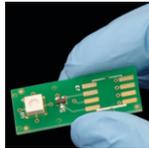
The **TruelabTM** Real Time micro PCR System enables decentralization and near patient diagnosis of Chikungunya by making real time PCR technology rapid, simple, robust and user friendly and offering "sample to result" capability even at resource limited settings. This is achieved through a combination of lightweight, portable, mains / battery operated **TruelabTM** Real Time micro PCR Analyzer and **TruenatTM MAG/AUTO** Sample Prep Device and room temperature stable **TruenatTM** chip-based Real Time PCR test and **TruenatTM MAG / AUTO** 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.

TruenatTM Chikungunya is a disposable, room temperature stable, chip-based Real Time PCR test with dried MgCl₂ in reaction well and freeze dried RT PCR reagents in microtube for performing a Real Time PCR test for detection of Chikungunya and runs on the **TruelabTM** Real Time 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 including standard value for quantitation. The **TruenatTM Chikungunya** chip also stores information of used test to prevent any accidental re-use of the test.

NOTE : **TruelabTM Uno / TruelabTM Uno Dx / TruelabTM Duo / TruelabTM Quattro / TruenatTM MAG / TruenatTM MAG** are all registered trademarks of Molbio Diagnostics (P) Limited.

The **TruelabTM** Real Time micro PCR Analyzer is protected by the following patents and patents pending: IN 2313/CHE/2007, WO 2009/047804 and corresponding claims of any foreign counterpart(s) thereof.

The **TruenatTM** 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

TruenatTM Chikungunya works on the principle of Real Time Reverse Transcriptase Polymerase Chain Reaction (RT PCR). The RNA from the patient blood sample is first extracted using **TruenatTM MAG** Sample Prep Device and **TruenatTM MAG** Blood Sample Prep Kit or using **TruenatTM MAG** Universal Cartridge based Sample Prep Device and **TruenatTM MAG** Universal Cartridge based Sample Prep Kit. The **TruenatTM Chikungunya** chip is placed on the chip tray of the **TruelabTM** 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 **TruenatTM Chikungunya** chip and the test is inserted in the **TruelabTM** 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 **TruenatTM Chikungunya** chip-based Real Time PCR test 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). Ct value is linearly correlated with the amount of target RNA copies present in the sample, enabling quantitative estimation of the analyte. Standard values for every batch are present in the **TruenatTM Chikungunya** chip and the analyzer automatically compares these with the Ct value of test sample to provide a quantitative result. 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, Chikungunya "DETECTED" or "NOT DETECTED" result is displayed and in positive cases, Ct values and Viruses per milliliter (Viruses/ml) is 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 **TruelabTM** micro PCR printer or transferred to the lab computer for any remote computer via Wifi network or 3G/GPRS network. Upto 5000 test results in **TruelabTM Uno** to 20000 results in **TruelabTM 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 Non-structural protein 4(*nsP4*) gene of the Chikungunya genome. The region selected is specific for Chikungunya virus.

5. CONTENT OF THE TruenatTM Chikungunya KIT

- A. Individually sealed pouches, each containing
1. **TruenatTM 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	601060005	601060020	601060050
	5T	20T	50T

6. CONTENTS OF THE TruenatTM AUTO Universal Sample Pre-treatment Pack (only for TruenatTM AUTO users)

- A. Lysis Buffer.
B. Disposable transfer pipette(graduated).

REF	60205AB05	60205AB20
	5T	20T

7. STORAGE AND STABILITY

TruenatTM 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 six (6) months at temperatures up to 40°C and one (1) month at temperatures up to 45°C. Avoid exposure to light or elevated temperatures (above recommended levels). Do not freeze.

TruenatTM 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

TruelabTM Real Time micro PCR Workstation (REF 603010001/62301001/633010001/64301001) consisting of

1. **TruenatTM MAG / AUTO** Sample Prep Device (REF 603040001/603041001).
2. **TruelabTM Uno / TruelabTM Uno Dx / TruelabTM Duo / TruelabTM Quattro** Real Time micro PCR Analyzer (REF 603020001/603021001/603022001/603023001).
3. **TruelabTM** micro PCR Printer (REF 603050001).
4. **TruepetTM** SPA fixed volume precision micropipette - 6 µl (REF 604070006).
5. **TruelabTM** Microtube Stand (REF 603070001).

Also required additionally are: **TruenatTM MAG** Blood Sample Prep Kit (REF 602010050)/**TruenatTM AUTO** Universal Cartridge Based Sample Prep kit (REF 60203AR05/REF 60203AR25/REF 60203AR50), **TruenatTM** Universal Control Kit (REF 601100008), DNase and RNase-free pipette tips with filter barrier, which may also be procured from **Molbio**, Powder free disposable gloves, waste disposal container with lid.

9. SPECIMEN PREPARATION FOR EXTRACTION WITH TruenatTM MAG

TruenatTM Chikungunya requires purified nucleic acids from whole blood/plasma collected in EDTA anticoagulant or serum specimen that are extracted using the **TruenatTM MAG** Sample Prep Device and **TruenatTM MAG** Blood Sample Prep kit (Refer to the User Manual of **TruenatTM MAG** Sample Prep Device and the package insert of **TruenatTM MAG** Blood Sample Prep kit for details).

10. SPECIMEN PREPARATION FOR EXTRACTION WITH TruenatTM AUTO

TruenatTM Chikungunya requires purified nucleic acids from whole blood/plasma collected in EDTA anticoagulant or serum specimen that are extracted using the **TruenatTM AUTO** Universal Cartridge based Sample Prep Device and **TruenatTM AUTO** Universal Cartridge based Sample Prep kit. Sample must be pre-treated using **TruenatTM 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.

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 40°C and 1 week at 30°C.

Nucleic acid extraction: Use entire content from the Lysis Buffer tube containing specimen for further procedure with the **TruenatTM AUTO** Universal Cartridge Based Sample Prep Device and **TruenatTM AUTO** Universal Cartridge Based Sample Prep Kit. (Refer to the User Manual of **TruenatTM AUTO** Universal Cartridge Based Sample Prep device and the package insert of **TruenatTM AUTO** Universal Cartridge Based Sample Prep kit for details). Dispose off lysis buffer tube after use, as per the section on "Disposal and Destruction" (Section 18).

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 **TruelabTM** 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 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 **TruenatTM Chikungunya** 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.

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 **TruenatTM** 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 **TruenatTM** assay reports "Not Detected" cannot be concluded to be negative for the concerned pathogen. As with any diagnostic test, results from the **TruenatTM** 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 **TruelabTM Uno / TruelabTM Uno Dx** Real Time micro PCR Analyzer user manual)

- Switch on the **Truelab™** Analyzer.
- If using the **Truelab™ Uno** device, also switch on the touch screen. If using the **Truelab™ Uno Dx/Duo/Quattro** proceed to step 3.
- Select User and enter password.
- For **Truelab™ Uno/Uno Dx**, select the test profile for "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 "Chikungunya" to be run from the Profiles Screen on the Analyzer screen.
- Enter the patient details as prompted in the **Truelab™** Analyzer screen.
- Press Start Reaction.
- For **Truelab™ Uno/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.
- Open a pouch of **Truenat™ Chikungunya** and retrieve the micro PCR chip, microtube and DNase & RNase free pipette tip.
- Label the chip and the tube with the patient ID using a marker pen at the space provided on the back side of the chip and the space on the microtube label.
- Place the **Truenat™ 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.
- 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 18). Using the filter barrier tip provided in the pouch, pipette out six (6) µL of the purified RNA from the Elute Collected 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™ 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 18).
- For **Truelab™ Uno/UnoDx**, slide the chip tray containing the **Truenat™ 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.
- Read the result from the screen.
- After the reaction is completed, for **Truelab™ Uno/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.
- Take out the **Truenat™ 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 18).
- 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).
- 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 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 would also display the Ct value and the Viruses per ml (Viruses/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 Universal Control kit (REF 601100008) 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.

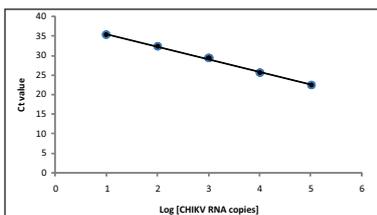
18. DISPOSAL AND DESTRUCTION

- Submerge the used **Truenat™ Chikungunya** chip, microtube, microtube cap, 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.
- Disinfect the solutions and/or solid waste containing biological samples before discarding them according to local regulations.
- 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).
- Do not autoclave materials or solutions containing sodium hypochlorite.
- 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:

Ct value 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 RNA dilutions were subjected to reverse transcription and the resulting cDNAs were tested using **Truenat™ Chikungunya** 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.



CLINICAL SENSITIVITY/ CLINICAL SPECIFICITY

Nineteen Chikungunya virus positive and 32 negative serum samples were processed using a commercial spin method to extract total RNA (as per manufacturer's protocol). Chikungunya analysis on **Truenat™ Chikungunya** was compared to the in-house standard Real time PCR assay of the **National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore**. The **Truenat™ Chikungunya** results for all 51 samples were found to be in agreement with the NIMHANS results i.e. all 32 negative samples were

undetected (specificity = 100%) and all 19 positive samples were detected as Chikungunya positive (sensitivity = 100%). The following table summarizes the results from the study.

PCR test on Truenat™ Chikungunya	PCR test at Hospital Lab		Total
	+ve	-ve	
+ve	19	0	19 (Truenat™ Chikungunya micro PCR +ve)
-ve	0	32	32 (Truenat™ Chikungunya micro PCR -ve)
Total	19 (In house standard real time PCR +ve)	32 (In house standard real time PCR -ve)	N=51

REPRODUCIBILITY AND PRECISION

Truenat™ Chikungunya kit was tested for reproducibility and precision between users, analyzers and reagent lots. Serial log dilutions of a high titre clinical specimen were used for this purpose. The same sample panel was provided to 3 users, who ran it on 3 different **Truelab™** Real Time micro PCR Analyzers with 3 different lots of **Truenat™ Chikungunya**. The tests were run in triplicates.

High inter-user, inter-analyzer and inter-lot reproducibility was observed (average standard deviation SD= 0.87Ct) and no significant difference was noted in the Ct values obtained from different users or analyzers or reagent lots.

INTERFERENCE

Effect of interferences of elevated blood or serum parameters such as lipid, cholesterol and triglycerides were evaluated in this study. Specimens (10 samples) showing higher level of serum parameters were spiked with known amounts of Chikungunya virus positive samples. These spiked samples were tested using the **Truenat™ Chikungunya**.

The Ct values obtained were found not to be significantly impacted (Ct Average = 28.47; standard deviation = 0.61, variance = 0.3; co-efficient of variation = 2%) by the presence of elevated biochemical parameters as described above.

Effect of elevated biochemical blood parameters:

Sr. No.	Sample I.D.	Cholesterol	HDL	Triglycerides	LDL	Ct
1.	ST1	230	38	259	140	29.50
2.	ST2	276	35	177	194	28.67
3.	ST3	225	32	353	114	28.21
4.	ST4	304	35	394	186	28
5.	ST5	168	32	342	58	28.5
6.	ST6	269	46	258	176	29
7.	ST7	220	42	230	124	27.33
8.	ST8	94	27	56	52	28.2
9.	ST9	225	39	138	148	28.33
10.	ST10	219	24	196	128	29

Carry Over Effect:

To determine whether the **Truenat™ Chikungunya** chip-based Real Time PCR test showed any signs of carryover of PCR products between runs, alternating runs of positive high titre samples and negatives samples were performed in duplicates. 5 positive samples and 5 negative samples were used for the study.

Results of carry-over evaluation

Sr No.	Sample I.D.	CT ST1	CT ST2	CT ST3	Average
1.	Positive	22.55	22.67	22.6	22.61
2.	Negative	-	-	-	-
3.	Positive	22.5	23.2	22.3	22.67
4.	Negative	-	-	-	-
5.	Positive	22.7	23.5	22.6	22.93
6.	Negative	-	-	-	-
7.	Positive	22.78	22.89	22.7	22.79
8.	Negative	-	-	-	-
9.	Positive	23.4	23.1	22.5	23.00
10.	Negative	-	-	-	-

All the positive samples were accurately detected and all negative samples were undetected indicating absence of carryover of PCR products between runs using the **Truenat™ Chikungunya** chip-based Real Time PCR test.

20. REFERENCES

- <http://www.cdc.gov/chikungunya>.
- Kularatne S.A.M. et al. (2009). Concurrent outbreaks of Chikungunya and Chikungunya fever in Kandy, Sri Lanka, 2006-07: a comparative analysis of clinical and laboratory features. *Postgrad Med J* 2009; 85:342-346.
- Mourya D.T., Thakare J.R., Gokhale M.D., et al. (2001) Isolation of chikungunya virus from Aedes aegypti mosquitoes collected in the town of Yawat, Pune District, Maharashtra State, India. *Acta Virol*; 45: 305-9.

SYMBOL KEYS

Consult instructions for use	IVD In vitro Diagnostic Medical Device. Not for medicinal use.	Temperature Limitation	Catalogue Number	For single use only	This Side Up	Manufacturer
Date of Manufacture	Date of Expiry	Batch Number / Lot Number	Caution	Contains sufficient for n-tests	EC REP	Authorised Representative in the European Community

molbio®

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