



Truenat®

Nipah

Chip-based Real Time PCR test for Nipah Virus

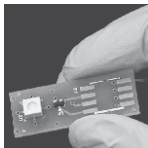
1. INTENDED USE

Truenat® Nipah (REF601390005 / 601390020 / 601390025 / 601390050 / 601390100 / 601390200) is a Chip-based Real Time Reverse Transcription Polymerase Chain Reaction (RT-PCR) test for semi-quantitative detection and the diagnosis of Nipah virus in Oropharyngeal swab and aids in the diagnosis of infection with Nipah virus RNA. **Truenat® Nipah** runs on the **Truelab®** Real Time Quantitative micro PCR Analyzers. **Truenat® Nipah** is an *in vitro* diagnostics test meant for professional use only.

2. INTRODUCTION

Nipah virus is a member of the family *Paramyxoviridae*, genus *Henipavirus*. Nipah virus was initially isolated and identified in 1999 during an outbreak of encephalitis and respiratory illness among pig farmers and people with close contact with pigs in Malaysia and Singapore. Its name originated from Sungai Nipah, a village in the Malaysian Peninsula where pig farmers became ill with encephalitis. Given the relatedness of Nipah virus to Hendra virus, bat species were quickly singled out for investigation and flying foxes of the genus *Pteropus* were subsequently identified as the reservoir for Nipah virus. The symptoms start to appear after 5–14 days from exposure. Initial symptoms are fever, headache, drowsiness followed by disorientation and mental confusion. These symptoms can progress into coma as fast as in 24-48 hours. Encephalitis, inflammation of the brain, is a potentially fatal complication of Nipah virus infection. Respiratory illness can also be present during the early part of the illness. Nipah-case patients who had breathing difficulty are more likely than those without respiratory illness to transmit the virus. The disease is suspected in symptomatic individuals in the context of an epidemic outbreak. Laboratory diagnosis of Nipah virus infection is made using reverse transcriptase polymerase chain reaction (RT-PCR) from throat swabs, cerebrospinal fluid, urine and blood analysis during acute and convalescent stages of the disease. IgG and IgM antibody detection can be done after recovery to confirm Nipah virus infection. Immunohistochemistry on tissues collected during autopsy also confirms the disease. Viral RNA can be isolated from the saliva of infected persons. In the 1999 outbreak, Nipah virus caused a relatively mild disease in pigs, but nearly 300 human cases with over 100 deaths were reported. In order to stop the outbreak, more than a million pigs were euthanized, causing tremendous trade loss for Malaysia. Since this outbreak, no subsequent cases (in neither swine nor human) have been reported in either Malaysia or Singapore.

The **Truelab®** Real Time micro PCR System enables decentralization and near patient diagnosis of Nipah 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 Kits so that even the peripheral laboratories with minimal infrastructure and minimally trained technician can easily perform these tests routinely in their facilities and report PCR results in less than an hour. Moreover, with these devices PCR testing can also be initiated in the field level, on site.



Truenat® Nipah 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 Real Time PCR test for detection and diagnosis of Nipah 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 including standard values for quantitation. The **Truenat® Nipah** chip also stores information of used test to prevent any accidental re-use of the test.

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® Nipah 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® Nipah** 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 PCR reagents, including reverse transcriptase (RT) 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® Nipah** 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® Nipah** Chip-based Real Time RT-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). 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 Nipah “DETECTED” or “NOT DETECTED” result is displayed and in positive cases, semi-quantitative result is also displayed on the screen. Based on the detection of the Internal Positive Control (IPC), the validity of the test run is also displayed. The IPC is a full process control that undergoes all the processes the specimen undergoes - from extraction to amplification thereby validating the test run from sample to result. Absence of or shift of IPC Ct beyond a pre-set range in case of negative samples invalidates the test run. While IPC will co-amplify in most positive cases also, in some specimen having a high target load, the IPC may not amplify, however the test run is still considered valid. The results can be printed via Bluetooth using the **Truelab® micro PCR** printer or transferred to the lab computer/or any remote computer via Wifi 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

The target sequence for this kit has been taken from the Nucleocapsid protein gene.

5. CONTENTS OF THE Truenat® Nipah KIT

- Individually sealed pouches, each containing
 - Truenat® Nipah** micro PCR chip
 - Microtube with freeze dried RT PCR reagents
 - DNase & RNase free pipette tip
 - Desiccant pouch
- Package Insert

REF	601390005	601390020	601390025	601390050	601390100	601390200
▽	5T	20T	25T	50T	100T	200T

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

- Lysis buffer
- Disposable transfer pipette(graduated)
- Package Insert

REF	60205AB05	60205AB20	60205AB25	60205AB50	60205AB100	60205AB200
▽	5T	20T	25T	50T	100T	200T

7. CONTENTS OF THE Trueprep® AUTO Transport Medium for Swab Specimen Pack

- Transport Medium for Swab specimen tubes (contains transport medium)
- Package Insert

REF	60206TS05	60206TS20	60206TS25	60206TS50	60206TS100	60206TS200
▽	5T	20T	25T	50T	100T	200T

8. STORAGE AND STABILITY

Truenat® Nipah chip is stable for six (6) months from the date of manufacture if stored between 2-30°C. Avoid exposure to light or elevated temperatures (above recommended levels). Do not freeze.

Trueprep® AUTO Transport Medium for Swab Specimen Pack and **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.

9. MATERIALS REQUIRED BUT NOT PROVIDED WITH THE KIT

Truelab® Real Time micro PCR Workstation (REF 623010001 / 633010001 / 643010001 / 653010001) consisting of

- Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device (REF 603041001 / 603042001)
- 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).

Also required additionally are: **Trueprep**® **AUTO** Universal Sample Pre-treatment Pack (REF 60205AB05 / REF 60205AB20 / REF 60205AB25 / REF 60205AB50 / REF 60205AB100 / REF 60205AB200), **Trueprep**® **AUTO** Transport Medium for Swab Specimen Pack (REF 60206TS05 / REF 60206TS20 / REF 60206TS25 / REF 60206TS50 / REF 60206TS100 / REF 60206TS200), **Trueprep**® **AUTO** Universal Cartridge Based Sample Prep Kit (REF 60203AR05 / REF 60203AR25 / REF 60203AR50 / REF 60203AR100) or **Trueprep**® **AUTO v2** Universal Cartridge Based Sample Prep Kit (REF 60207AR05 / REF 60207AR25 / REF 60207AR50 / REF 60207AR100), **Truenat**® Positive Control Kit - Panel IV (REF 801040008), Powder free disposable gloves and waste disposal container with lid.

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

Swab specimen:

Throat swab specimen must be collected as per standard procedures using a standard nylon flocked swab. Insert the swab with specimen into the Transport Medium for Swab Specimen Tube provided and mix well by repeatedly twirling the swab in the buffer solution. Gently break the handle of the nylon swab at the break point, leaving the swab containing the specimen in the Transport Medium for Swab Specimen Tube. Tightly close the cap of the Transport Medium for Swab Specimen Tube (Refer to the package insert of **Trueprep**® **AUTO** Transport Medium for Swab Specimen Pack for further details). ⚠ Dispose off the remaining part of the swab after use, as per the section on "Disposal and Destruction" (Section 18).

Sample Storage and Transportation:

Transport Medium for Swab Specimen decontaminates the specimen and makes it ready for storage/ transportation/ extraction. The specimen in this form is stable for up to three (3) days at 40°C and one(1) week at 30°C.

Nucleic acid extraction:

Transfer 500 µL from the Transport Medium for Swab Specimen Tube into the Lysis Buffer Tube for Oropharyngeal swab for further procedure (Refer to the package insert of **Trueprep**® **AUTO** Universal Sample Pre-treatment pack for further details) 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 Transport Medium for Swab Specimen Tube with cap, lysis buffer tube with cap and transfer pipette 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, 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.

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**® **Nipah** 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.

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-quantification 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 user and enter password.
3. For **Truelab**® **Uno Dx**, select the test profile for "Nipah" 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 "Nipah" 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**® **Nipah** and retrieve the Chip-based Real Time PCR test and the microtube.
8. Place the **Truenat**® **Nipah** chip on the chip tray without touching the white reaction well. The reaction well should be facing up and away from the Analyzer. Gently press the chip to ensure that it has seated in the chip tray properly.
9. Place the microtube containing freeze dried RT-PCR reagents in the microtube stand provided along with the **Truelab**® Real Time micro PCR workstation **after ensure that white pellet of dried PCR reagents remain 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 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**® **Nipah** 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**® **Nipah** 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**® **Nipah** 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 & INTERPRETATIONS

Two amplification curves are displayed on the **Truelab**® Real Time Quantitative micro PCR Analyzer screen to indicate the progress of the test. Either or 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 viral genomes 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 viral load as "HIGH (Ct<20)", "MEDIUM (20≤Ct<25)", "LOW (25≤Ct<30)" or "VERY LOW (Ct ≥ 30)" for positive specimen. The result screen also displays the validity of the test run as "VALID" or "INVALID". Invalid samples have to be repeated with fresh specimen from the sample preparation stage. *While IPC will co-amplify in most positive cases also, in some specimen having a high target load, the IPC may not amplify, however the

test run is still considered valid.

17. 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. **Truenat**[®] Positive Control Kit - Panel IV (REF 801040008) containing Positive Control and Negative Control must be ordered separately. It is advisable to run controls under the following circumstances. ● Whenever a new shipment of test kits is received. ● When opening a new test kit lot. ● If the temperature of the storage area falls outside of 2-30°C. ● By each new user prior to performing testing on clinical specimen.

18. DISPOSAL AND DESTRUCTION

1. Submerge the used content such as **Truenat**[®] **Nipah** chip, microtube, microtube cap, pipette tips, remaining part of nylon flocked swab, Transport Medium for Swab Specimen Tube, 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

Robustness:

Study was carried out to determine whether the **Truenat**[®] **Nipah** test showed any signs of carryover of PCR products between runs. Potential sample carryover was evaluated by testing alternate positive followed by negative samples. The number of samples run were 12 positives and 12 negatives. The **Truenat**[®] **Nipah** test did not exhibit detectable carryover contamination from positive to negative samples.

Reproducibility:

Study was carried out to compare the functional performance of the **Truenat**[®] **Nipah** assay using three different titres of 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 as Inter User (1.22), Inter day (1.36) and Inter Device (0.54) which were in the accepted range of ≤15% CV for **Truenat**[®] **Nipah** assay

Precision:

Precision was tested by performing **Truenat**[®] **Nipah** assay with extracted RNA of High (1.0E+05 copies/mL), Medium (1.0E+04 copies/mL) and Low (1.0E+03 copies/mL) titres of RNA for five consecutive days. Every day PCR for each titre RNA was run in duplicates. The %CV values obtained for High titre (1.27), Medium titre (0.99) and low titre (0.99) were within the accepted range of ≤15% CV for **Truenat**[®] **Nipah** assay.

Analytical sensitivity:

Evaluation of analytical sensitivity or limit of detection of **Truenat**[®] **Nipah** assay, in comparison to TaqMan NiV qRT-PCR assay was performed. Nipah tissue culture derived virus stock with known TCID₅₀ titre of 10^{5.5}/ml was serially diluted 10-fold upto 10⁻¹⁰ dilution. All three lots of **Truenat**[®] **Nipah** chips could detect the Nipah virus TCID₅₀ stock upto the same dilution (TCID₅₀ 10^{1.17}/ml) equivalent to 10 RNA copies/ml.

Linearity of assay:

The linearity of the **Truenat**[®] **Nipah** assay was evaluated by testing 10 fold serial dilutions of Nipah virus tissue culture fluid. The assay was found to be linear over the range from 10^{7.17} to 10^{1.17} TCID₅₀ titre/mL tested and the R² was found to be 0.997.

Clinical Evaluation 1:

A detailed clinical evaluation was conducted at ICMR-NIV in Pune to determine the sensitivity and specificity of the **Truenat**[®] **Nipah** assay. The results of the **Truenat**[®] **Nipah** assay were compared with those of the NIV lab's standard TaqMan Real Time PCR in a series of tests using Nipah-positive human specimens collected from outbreak-affected areas, experimental tissue specimens of bats and hamsters, and confirmed Nipah-negative human and bat clinical specimens. It was found that the results of these tests were concordant.

Clinical sensitivity:

A total of 65 clinical samples which included human specimens (n=20) such as throat

swabs, serum and EDTA blood spiked with the Nipah virus stock of known TCID₅₀, bat necropsy organs specimens (n=15) and experimentally infected hamster organ specimens (n=30) were tested using all the 3 lots of **Truenat**[®] **Nipah** microchips and gold standard TaqMan real time RT-PCR simultaneously. Similarly, a total of 39 negative human specimens (n=16) including EDTA blood, serum, TS/NS and Bat necropsy organs (n=13) when extracted by **Trueprep**[®] **AUTO** extraction method as well as Magmax extraction method were tested using all the 3 lots of **Truenat**[®] **Nipah**. All the 3 **Truenat**[®] **Nipah** lots showed 100% sensitivity as compared with the gold standard TaqMan Nipah Real Time RT-PCR.

Clinical specificity:

To evaluate the cross-reactivity using the 3 lots of **Truenat**[®] **Nipah** microchips, samples positive for Measles virus (Paramyxoviridae), Influenza virus (Orthomyxoviridae family), SARS CoV-2 (coronavirus), Dengue virus (Flaviviridae), Chikungunya and Rubella viruses (Togaviridae), Herpes Simplex virus (HSV) and Varicella Zoster virus (VZV) were tested by the **Truenat**[®] **Nipah** POCT assay. A total of 49 clinical specimens of humans when tested comparatively using the 3 different lots of **Truenat**[®] **Nipah**, showed 100% specificity with no cross reactivity with 7 different family of viruses.

Clinical Evaluation 2:

A total of 10 earlier tested positive clinical specimens RNA were tested by **Truenat**[®] **Nipah** assay gave 100% concordant results.

Inter-machine variation

NiV Positive control RNA extracted by both **Trueprep**[®] **AUTO** RNA extraction units and tested on all the 5 **Truelab**[®] **Uno Dx** units were successfully detected with no discrepancy in results and Ct values were also found to be comparable. Also NiV positive control RNA of known Ct value on all 5 **Truelab**[®] PCR machines, thereby ruling out inter-machine variation and establishing concordancy in the functioning of the **Truelab**[®] PCR machine software algorithm.














Inactivation Efficacy of Trueprep[®] AUTO Universal Sample Pre-treatment Pack Lysis buffer

The re-suspended pellet infected VeroCCL81 cells did not show any CPE upto 2 blind passages and till 7 PID in each passage. On the other hand, virus control indicated CPE on 1 PID and cell control flasks did not show any CPE at all. Further TaqMan based Real time RT-PCR results of the cell culture samples confirmed the inactivation of the Nipah virus in the lysis buffer treated and re-suspended pellet.

20. REFERENCES

1. Cocker et al.(2011). Emerging infectious diseases in southeast Asia: regional challenges to control. *Lancet*. 377: 599–609.
2. Luby SP (2013). The pandemic potential of Nipah virus. *Antiviral Research*. 100: 38–43.
3. Chua KB et al. (2000). Nipah virus: a recently emergent deadly paramyxovirus. *Science*. 288: 1432-35.
4. WHO Nipah Fact sheets.(2018). <https://www.who.int/news-room/fact-sheets/detail/nipah-virus>.
5. Chadha MS et al. (2006). Nipah Virus-associated Encephalitis Outbreak, Siliguri, India. *EID*.2006; 12(2), 235-240.
6. Arankale VA et al. (2011). Genomic Characterization of Nipah Virus, West Bengal, India. *Emerg Infect Dis*. 17(5):907-909.

SYMBOL KEYS

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



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