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Truenat®

Nipah

Chip-based Real Time PCR test for Nipah Virus

1. INTENDED USE

REF **Truenat® Nipah (REF601390005 / 601390020 / 601390025 / 601390050 / 601390100 / 601390200)** is an automated point-of-care or near patient 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 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.

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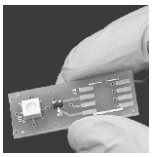
2. INTRODUCTION

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 **Truenat®** point-of-care real time PCR system enables decentralization and near patient diagnosis of Nipah 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®** 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 $MgCl_2$ 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. All 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® 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 patient sample (oropharyngeal swab specimen) is first pre-treated using the **Trueprep® AUTO** Universal Sample Pre-treatment Pack. 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 cartridge from the **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit contains pre-loaded Internal Positive Control (IPC), composed of known concentration of DNA, trehalose, PBS buffer and amaranth dye, which is co-extracted along with sample nucleic acids, thereby validating the process from extraction to PCR run. 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 calibrated 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 4G/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. CONTENT OF THE Truenat® Nipah KIT

A. Individually sealed pouches
B. Package Insert

Each individually sealed pouch contains:

1. **Truenat® Nipah** micro PCR chip (1 No.)
2. Microtube with freeze dried RT-PCR reagents (1 No.)
3. DNase and RNase free pipette tip (1 No.)
4. Desiccant pouch (1 No.)

Pack sizes of **Truenat® Nipah**

REF	601390005	601390020	601390025	601390050	601390100	601390200
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.

Pack sizes of **Trueprep® AUTO Universal Sample Pre-treatment Pack**

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

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

A. Transport Medium for Swab specimen tubes (contains transport medium).
B. Package Insert.

Pack sizes of **Trueprep® AUTO Transport Medium for Swab Specimen Pack**

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

8. 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

9. STORAGE, HANDLING AND STABILITY


1. **Truenat® Nipah** test 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.


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.

10. 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 (REF603041001/603042001).
2. **Truelab® Uno Dx/Truelab® Duo/Truelab® Quattro** Real Time micro PCR Analyzer (REF603021001/603022001/603023001).
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 / 60205AB20 / 60205AB25 / 60205AB50 / 60205AB100 / 60205AB200), **Trueprep® AUTO** Transport Medium for Swab Specimen Pack (REF 60206TS05 / 60206TS20 / 60206TS25 / 60206TS50 / 60206TS100 / 60206TS200), **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), The **Truenat®** Positive Control Kit - Panel IV (REF 801040008), powder free disposable gloves, waste disposal container with lid and sodium hypochlorite.

11. 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 19).

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 40ºC and one(1) week at 30ºC.

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 19).

12. 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.

IVD 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.

13. 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.
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.

14. 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.

15. 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.

16. 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 "Nipah" 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 "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 micro PCR chip, microtube and DNase and RNase free pipette tip. Do not open the pouch until ready to test.
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 place the chip on the chip tray by aligning it in the slot provided.
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 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 19). 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® 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 19).
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 "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® Nipah** micro PCR chip at end of the test and dispose it off as per the section on "Disposal and Destruction" (Section 19).
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 **Truelab®** analyzer manual).
15. Switch off the **Truelab®** analyzer.

17. RESULTS AND 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.

18. 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.

19. 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.

20. 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 R^2 was found to be 0.997.

Clinical Evaluation:
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 Simples 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:
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 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.

21. REFERENCES

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SYMBOL KEYS

Consult instruction for use.	IVD *In vitro* Diagnostic Medical Device. Not for medicinal use.	LOT Batch number/ Lot number.	REF Catalogue number.	UDI Unique Device Identifier.	This way up.	Manufacturer.	Caution.	NON STERILE Non sterile.
Contains sufficient for <n> tests	Temperature limitations.	Date of manufacture.	Date of expiry.	For single use only.	Keep dry.	Keep away from sunlight	EC REP Authorised Representative in European Community	Device for near-patient testing

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