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Truenat®

Rabies

Chip-based Real Time PCR Test for Rabies Virus

1. INTENDED USE

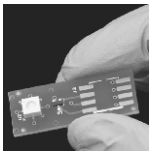
REF **Truenat® Rabies (REF 601120005 / 601120020 / 601120025 / 601120050 / 601120100 / 601120200)** is an automated point-of-care or near patient Chip-based Real Time Reverse Transcription Polymerase Chain Reaction (RT-PCR) test for the semiquantitative detection of Rabies virus infection in a variety of specimens including saliva, cerebrospinal fluid (CSF), brain tissue from animals. Additionally, it aids in the diagnosis of Rabies virus infection in human patients through the analysis of brain specimens and antemortem specimens such as saliva, CSF, and nuchal skin biopsy. **Truenat® Rabies** runs on the **Truelab®** Real Time Quantitative micro PCR Analyzers. **Truenat® Rabies** 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.

IVD

2. INTRODUCTION

Rabies is a zoonotic disease which affects the central nervous system causing acute encephalitis in warm blooded animals. The disease is primarily transmitted through the bite of a rabid animal. The Rabies virus uses the peripheral nerves to travel to the brain. Once the infection reaches the central nervous system, the infection becomes non treatable leading to death within a short span. As per WHO sources, there are an estimated 60,000 human deaths annually from Rabies worldwide. Of these deaths 45-50% occurs in India. Animal bites in India amounts to nearly 15 million/year, of that a major portion being dog bites. This has resulted in India having the highest number of human Rabies deaths. Since Rabies is not a notifiable disease in India and there is no organized surveillance system, the actual number of deaths may be much higher.

Since the 1960s, the standard test for Rabies has been Direct Fluorescent Antibody test (DFA test). Because Rabies is present in nervous tissue (and not blood like many other viruses), it is best to test for Rabies in brain tissue. This test can only be done post-mortem. In living beings, several tests are required to diagnose Rabies because no single test is sufficient. The diagnosis of Rabies is routinely based on clinical and epidemiological information, especially when exposures are reported in Rabies-endemic countries. Established diagnostic techniques include the direct fluorescent antibody test, mouse inoculation test, (MIT) and the Rabies tissue culture inoculation test (RTCIT). Diagnostic tests using conventional assays that appear to be negative, even when undertaken late in the disease and despite the clinical diagnosis, have a tendency, at times, to be unreliable. These tests are rarely optimal and entirely dependent on the nature and quality of the sample supplied. In the course of the past three decades, the application of molecular biology has aided in the development of tests that result in a more rapid detection of Rabies virus. These tests enable viral strain detection from clinical specimens. Currently, there are a number of molecular tests that can be used to complement conventional tests in Rabies diagnosis. However molecular tests have so far been restricted to centralized reference laboratories as they require skilled manpower and elaborate infrastructure. Also, the turnaround time for results could take a few days.



The **Truenat®** point-of-care real time PCR System enables decentralization and near patient diagnosis of and monitoring of Rabies. This is enabled by making the 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® Rabies is a disposable, room temperature stable, chip-based Real Time RT PCR test with dried MgCl2 in reaction well and freeze-dried RT PCR reagents in microtube for performing Real Time RT-PCR test for viral infection and runs on the **Truelab®** Real Time micro-PCR Analyzer. All the components of **Truenat®** pouch are nuclease-free. It requires only six (6) µL of purified RNA to be added to the reaction well for the analysis. The intelligent chip also carries test and batch related information. The **Truenat® Rabies** chip also stores information of used test to prevent any accidental re-use of the chip.

NOTE :Truelab®/ Truenat® / Trueprep® / Truepet® are all trademarks of Molbio Diagnostics Private Limited.

The Truelab® Real Time micro PCR Analyzer is protected by the following patents and patents granted: IN 2313/CHE/2007 (Patent No. 281573), WO2009/047804 and corresponding claims of any foreign counterpart(s) thereof.

The Truenat® micro PCR chip is protected by the following patents and patents pending: IN 2312/CHE/2007, WO 2009/047805 and corresponding claims of any foreign counterpart(s) thereof.

3. PRINCIPLE OF THE TEST

Truenat® Rabies works on the principle of Real Time Reverse Transcription Polymerase Chain Reaction (RT PCR) based on Taqman chemistry. The patient sample (saliva / cerebrospinal fluid (CSF) / brain tissue / nuchal skin biopsy) 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), it is a synthetic plasmid construct provided in a stable formulation preloaded into Cartridges which is co-extracted along with sample nucleic acids, thereby validating the process from extraction to PCR run. The RNA extract is analyzed using the **Truenat® Rabies** Chip-based Real Time Reverse Transcription Polymerase Chain Reaction (RT-PCR) test and the **Truelab®** Real Time Quantitative micro PCR Analyzer. The **Truenat® Rabies** chip is placed on the chip tray of the **Truelab®** Real Time Quantitative micro PCR Analyzer. Six (6) µL of the purified RNA is then dispensed using the provided calibrated micropipette and tip into the microtube containing freeze dried RT-PCR reagents and allowed to stand for 30-60 seconds to get a clear solution. ⚠ No mixing by tapping, shaking or by reverse pipetting should be done. Six (6) µL of this clear solution is then pipetted out using the same pipette and tip and dispensed into the reaction well of the **Truenat® Rabies** chip and the test is inserted in the **Truelab®** Real Time Quantitative micro PCR Analyzer where the RNA is first converted into complementary DNA (cDNA) by the RT enzyme and further thermal cycling takes place. A positive amplification causes the dual labeled fluorescent probe in the **Truenat® Rabies** chip to release the fluorophores in an exponential manner and the emitted light is then captured by the built-in opto-electronic sensor and displayed as amplification curve on the analyzer screen, on a real time basis during the test run. The Cycle threshold (Ct) is defined as the number of amplification cycles required for the fluorescent signal to cross the threshold (i.e. exceed the background signal). Ct levels are inversely proportional to the amount of target nucleic acid in the sample. (i.e. the lower the Ct level the greater is the amount of target nucleic acid in the sample). In the case of negative samples, amplification does not occur and a horizontal amplification curve is displayed on the screen during the test run. At the end of the test run, a Rabies"DETECTED" or "NOT DETECTED" result is displayed and in positive cases, Ct values and Copies/ml is also displayed on the screen. Based on the Ct of the internal positive control (IPC), the validity of the test run is also displayed. The IPC is a full process control that undergoes all the processes the specimen undergoes - from extraction to amplification thereby validating the test run from sample to result. Absence of or shift of IPC Ct beyond a pre-set range in case of negative samples invalidates the test run. While IPC will co-amplify in most positive cases also, in some specimen having a high target load, the IPC may not amplify, however the test run is still considered valid. The results can be printed via Bluetooth using the **Truelab®** micro PCR printer or transferred to the lab computer/or any remote computer via Wifi network or 4G/3G/GPRS network. Upto 20000 results in **Truelab® Uno Dx/Duo/Quattro** can be stored on the analyzer for future recall and reference.

4. TARGET SELECTION

The target sequence for this assay is the nucleoprotein gene of the Rabies genome.

5. CONTENT OF THE Truenat® Rabies KIT

A. Individually sealed pouches
B. Package Insert

Each individually sealed pouch contains:

1. **Truenat® Rabies** 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® Rabies**

REF	601120005	601120020	601120025	601120050	601120100	601120200
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® Rabies** 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 and **Trueprep® AUTO** Transport Medium for Swab Specimen Pack are 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 sunlight.
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).
6. Dry Bath (for Rapid PCR protocol)

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

Truenat® Rabies requires purified nucleic acids from saliva, cerebrospinal fluid (CSF), brain tissue and nuchal skin biopsy specimens 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 (Refer to the User Manual of **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device and the package inserts of **Trueprep® AUTO** Transport Medium for Swab Specimen Pack, **Trueprep® AUTO** Universal Sample Pre-treatment Pack, **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Kit for details).

Nucleic acid extraction:

Brain Tissue and Nuchal skin biopsy: Take 100 mg of the brain tissue or nuchal skin biopsy, in a micro tube. To the tube 100 µL of lysis buffer from **Trueprep® AUTO** Universal sample pre-treatment pack is added and tissue is homogenized with micro pestle. After homogenization the entire content is then transferred to the remaining lysis buffer tube and it is allowed to stand for 5 minutes. The entire content of lysis buffer tube containing the specimen is subjected to 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).

For saliva and CSF: Transfer 0.5 ml of saliva or CSF using the transfer pipette provided into the lysis buffer tube provided and mix well. Use the entire content of lysis buffer tube containing the 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 Sample Prep Kit for details).

12. SAFETY PRECAUTIONS

IVD 1. For *in vitro* diagnostic use only.
2. Bring all reagents and specimen to room temperature (20-30°C) before use.
3. Do not use kit beyond expiry date.
4. Carefully read the user manuals, package inserts and Material Safety Data Sheets (MSDS) of all the components of the **Truenat®** point-of-care real time PCR system before use.
5. Good laboratory practices are recommended to avoid contamination of specimens or reagents.
6. All materials of human origin should be handled as though potentially infectious.
7. Do not pipette any material by mouth.
8. Do not eat, drink, smoke, apply cosmetics or handle contact lenses in the area where testing is done.
9. Use protective clothing and wear disposable gloves when handling samples and while performing sample extraction.
10. Do not substitute assay components / reagents with any other components / reagents.
11. Each single-use **Truenat®** chip is used to process one test. Do not reuse chip.

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 vapors (e.g., from Sodium hypochlorite, acids, alkalis or aldehydes) or dust.
3. While retrieving the Truenat® Rabies micro-PCR 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.

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 bio-hazard 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 "Rabies" 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 "Rabies" 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® Rabies** 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® Rabies** 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® Rabies** 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® Rabies** 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® Rabies** 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 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 virus copies 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 also display the viral load as "HIGH", "MEDIUM", "LOW" or "VERY LOW" 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 micro PCR Analyzer is working accurately, run positive and negative controls from time to time. The **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 **Truenat® Rabies** chip, microtube, microtube cap, transfer pipette, pipette tips, nylon flocked swab, Sample pre-treatment tube, 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

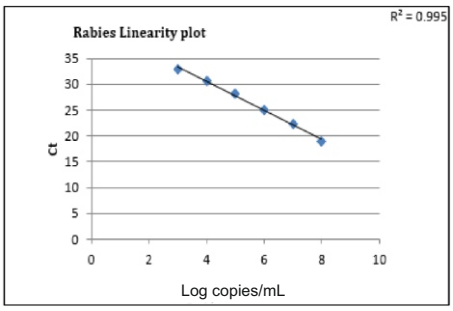
Analytical Exclusivity (Primer Specificity): The following microorganisms were evaluated in silico from the NCBI database using the NCBI nucleotide blast and primer blast tools to determine for potential cross-reactivity in the **Truenat® Rabies** assay.

Organisms	Organisms
Human DNA	Vaccinia virus
Herpes Simplex Virus-1	Alphapapillomavirus 7
Herpes Simplex Virus-2	Alphapapillomavirus 9
SARS COV 2	Human herpes virus 8
Human immunodeficiency virus (1& 2)	*E. coli*
Hepatitis B virus	*Staphylococcus epidermidis*
Hepatitis C virus	*Mycobacterium tuberculosis*
Parvovirus	*Mycobacterium gordonae*
Adenovirus	*Neisseria gonorrhoeae*
Cytomegalovirus	*Chlamydia trachomatis*
Influenza B Virus	*Candida albicans*
Human herpes virus 3	*Staphylococcus aureus*
Human herpesvirus 4	*Enterobacter aerogenes*
Human herpesvirus 6	*Klebsiella pneumoniae*

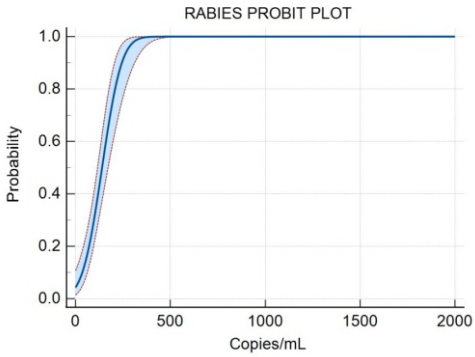
Linearity:

The linearity analysis was performed according to CLSI Guidelines. Serial dilutions of the Rabies IVT RNA was made from 1.00E+08 copies/mL to 1.00E+03 Copies/mL and nucleic acids were extracted on **Trueprep® AUTO**

Universal Cartridge Based Sample Prep Device in triplicates for each dilution followed by PCR on **Truelab**® analyzer using **Truenat**® **Rabies** test. The assay is found to be linear over 6 orders of magnitude (from 1.00E+08 to 1.00E+03 copies/mL) for Rabies RNA.



Limit of detection (LoD):
The LoD was determined by testing dilutions of Rabies IVT RNA in saliva matrix and performing nucleic acid extractions on**Trueprep**® **AUTO** Universal Cartridge Based Sample Prep Device for each of the dilution 24 times followed by PCR on **Truelab**® analyzer. Probit analysis of the data was used to determine the concentration of the RNA with 95% probability of detection. The LoD was determined to be 273.83 copies/mL for Rabies by **Truenat**® **Rabies** assay.



LoD: 273.83 copies/mL [95% CI: 229 – 352]

Robustness:
Potential sample carryover within the **Truenat**® **Rabies** test was evaluated by testing alternate positive followed by negative samples. The number of samples run was 20 positives and 20 negatives. The results showed no carryover contamination. The **Truenat**® **Rabies** test did not exhibit detectable carryover contamination from positive to negative samples.

Interference:
To determine the effect of interfering substances on the **Truenat**® **Rabies** one negative and one positive sample is used. The sample matrix used was saliva. To the sample different concentrations of interfering substances were spiked into the respective interfering substances used as: Human DNA: 0.4 mg/dL, Hemoglobin: 500 mg/dL, Billirubin: 20 mg/dL, Triglycerides: 3 mg/dL, Serum albumin: 9 g/dL, PEP: 100 µg/mL, KedRAB: 1 mg/mL, BayRab: 1 mg/mL, Imovax: 1 mg/mL, Rabavert: 100 µg/mL and then the samples were subjected to sample prep on **Trueprep® AUTO** Universal Cartridge Based Sample Prep Device. RNA was eluted and PCR was performed on **Truelab**® devices using **Truenat**® **Rabies** tests. The presence of the above mentioned potential interference substances did not interfere with the performance of **Truenat**® **Rabies** assay.

Reproducibility:

Precision:
Precision was tested by performing **Truenat**® **Rabies** test with three different titres of RNA for five consecutive days. Every day PCR for each titre of RNA was run in triplicates. The %CV values obtained for Titre 1 (1.01), Titre 2 (1.33) and Titre 3 titre (1.18) were within the accepted range of ≤15% CV for **Truenat**® **Rabies** assay.

Clinical validation:
Comprehensive evaluation of **Truenat**® **Rabies** in comparison to reference FAT in Animal Specimen and comparison to FAT and Routine Real time RT PCR for detection of Rabies in Human Specimen was conducted at investigation site ,Department of Neurovirology, NIMHANS Bangalore.

Animal Specimens:
A total of 149 animal brain samples were used for testing on **Truenat**® **Rabies** assay and compared against Fluorescent Antibody Test (FAT) as the reference test. All animal brain tissues were subjected to FAT for detection of rabies nucleoprotein antigen by subjecting smears made from cut surfaces of fresh brain tissues, fixing in cold acetone for 2 hrs, air drying and then treating with a cocktail of anti-rabies monoclonal antibodies conjugated with FITC for 30 min at 37°C in an incubator in a humid chamber. For **Truenat**® **Rabies** assay, all animal brain tissues were homogenized, and five hundred microliters of homogenized specimen were used for the automated extraction and **Truenat**® loading procedure as per protocol. The **Truenat**® results of canine brain specimens were compared with the FAT results, and the sensitivity of **Truenat**® was found to be 100.00% (95% confidence interval [CI]: 97.07%-100.00%), specificity was 86.96% (95% CI: 66.41%- 97.22%), and diagnostic accuracy was 97.96% (95% CI: 94.15%-99.58%).

Human Specimens:
In a comprehensive study, both postmortem (brain tissue from suspect cases and Human Brain Tissue Repository (HBTR)) and antemortem (Saliva, CSF, Nuchal skin biopsy) human specimens, were 1) 48 no. of samples were run in parallel using **Truenat**® **Rabies** and its performance was compared with fluorescent antibody test (FAT) as the reference test. A total of 48 specimen were tested which yielded valid **Truenat**®results and on comparison with FAT, the sensitivity of **Truenat**® was found to be 100 % (95% CI 87.23%-100%) and the specificity was found to be 100 % (95% CI 83.89%-100%) and 2) 152 no. Of samples were run in parallel using **Truenat**® **Rabies** and its performance was compared with Routine Rabies RT PCR (Qiagen). The **Truenat**® **Rabies** positivity rate was higher than routine RT PCR for all human antemortem specimens combined (18.42% vs.14.47%).

21. REFERENCES

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22. REVISION HISTORY

Section	Description of the changes
1	Intended use is updated
3	Principle of the test updated
11	section 11 is updated to remove sample storage and transportation

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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Molbio Diagnostics Private Limited

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

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

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