CE

Truenat®

Rabies

Chip-based Real Time PCR Test for Rabies Virus

1. INTENDED USE

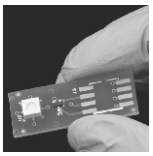
Truenat® Rabies (REF 601120005 / 601120020 / 601120025 / 601120050 / 601120100 / 601120200) is a chip-based Real Time Reverse Transcription Polymerase Chain Reaction (RT PCR) test for the semi quantitative detection of Rabies virus in Cerebrospinal fluid (CSF), saliva, brain tissue, corneal swabs specimens of animals and aids in the diagnosis of infection with Rabies virus. **Truenat® Rabies** runs on the **Truelab®** Real Time Quantitative micro PCR Analyzers.

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.

There are an estimated 55,000 human deaths annually from rabies worldwide. Of these deaths 45- 50% occur in India. Animal bites in India amounts to nearly 15 million/year. 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 identification 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 **Truelab®** Real Time micro PCR System enables decentralization and near patient diagnosis of Rabies 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 micro PCR Analyzer and **Trueprep® AUTO / AUTO v2** Universal Cartridge Based Sample Prep Device and room temperature stable **Truenat®** micro PCR chips and 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, micro PCR test with dried $MgCl_2$ in reaction well and freeze dried RT PCR reagents in microtube for performing Real Time RT-PCR test for Rabies virus and runs on the **Truelab®** Real Time Quantitative micro PCR Analyzer. It requires only six (6) µL of purified RNA to be added to the reaction well for the analysis. The intelligent chip also carries test and batch related information. The **Truenat® Rabies** chip also stores information of used chips to prevent any accidental re-use of the chip.

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 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® Rabies** chip is placed on the chip tray of the **Truelab®** Real Time micro PCR Analyzer. Six (6) µL of the purified RNA is then dispensed using the provided micropipette and tip into the microtube containing freeze dried RT PCR reagents and allowed to stand for 30-60 seconds to get a clear solution. ⚠ No mixing by tapping, shaking or by reverse pipetting should be done. Six (6) µL of this clear solution is then pipetted out using the same pipette and tip and dispensed into the reaction well of the **Truenat® 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 labeled fluorescent probes in the **Truenat® Rabies** chip to release the fluorophores in an exponential manner which is then captured by the built-in opto-electronic sensor and displayed as amplification curve on the analyzer screen, on a real time basis during the test run. The Cycle threshold (Ct) is defined as the number of amplification cycles required for the fluorescent signal to cross the threshold (i.e. exceed the background signal). Ct levels are inversely proportional to the amount of target nucleic acid in the sample. (i.e the lower the Ct level the greater is the amount of target nucleic acid in the sample). In the case of negative samples, amplification does not occur and a horizontal amplification curve is displayed on the screen during the test run. At the end of the test run, Rabies "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 using the **Truelab® micro PCR printer** or transferred to the lab computer/or any remote computer via Wifi network or 3G/GPRS network. Upto 20,000 results in **Truelab® Uno Dx / 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, each containing
   1. **Truenat® Rabies** micro PCR chip.
   2. Microtube with freeze dried RT PCR reagents.
   3. DNase & RNase free pipette tip.
   4. Desiccant pouch

B. Package Insert

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

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

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

8. STORAGE AND STABILITY

Truenat® Rabiesis stable for two (2) years from the date of manufacture if stored between 2-30°C. It is also stable for upto one (1) month at temperatures up to 45°C. Avoid exposure to light or elevated temperatures (above recommended levels). Do not freeze.

Trueprep® AUTO Universal Sample Pre-Treatment Pack and **Trueprep® AUTO** Transport Medium for Swab Specimen 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

1. **Trueprep® AUTO/AUTO v2** Universal Cartridge Based Sample Prep Device (REF603041001 / 603042001).
2. **Truelab® Uno Dx / Truelab® Duo / Truelab® Quattro** Real Time micro PCR Analyzer (REF 603021001 / 603022001 / 603023001).
3. **Truelab®** micro PCR Printer (REF 603050001).
4. **Truepet®** SPA fixed volume precision micropipette - 6 µl (REF 604070006).
5. **Truelab®** Microtube Stand (REF 603070001).

Also required additionally are: **Trueprep® AUTO** Universal Sample Pre-treatment Pack (REF60205AB05 / REF60205AB20 / REF60205AB25 / REF60205AB50 / REF60205AB100 / REF60205AB200), **Trueprep® AUTO** Transport Medium for Swab Specimen Pack (REF60206TS05 / REF60206TS20 / REF60206TS25 / REF60206TS50 / REF60206TS100 / REF60206TS200), **Trueprep® AUTO** Universal Cartridge Based Sample Prep Kit (REF60203AR05 / REF60203AR25 / REF60203AR50 / REF60203AR100) or **Trueprep® AUTO v2** Universal Cartridge Based Sample Prep Kit (REF60207AR05 / REF60207AR25 / REF60207AR50 / REF60207AR100), **Truenat®** Positive Control Kit - Panel IV (REF 801040008), Powder free disposable gloves, waste disposal container with lid.

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

Pretreatment for Brain Tissue: Take 100mg of the brain tissue, add 100µL of VLM and homogenize using a micro pestle into a fine suspension. After homogenization add 900µL of additional VLM. The volume of the suspension will be 1ml. Take the entire 1ml suspension and subject to nucleic acid extraction on **Trueprep® AUTO / AUTO v2** extraction system.

For Saliva: Take 0.5 ml of saliva sample and subject to nucleic acid extraction on **Trueprep® AUTO / AUTO v2** extraction system.

For Corneal swabs: Collect the Corneal swabs in 1ml VLM and the entire 1ml VLM is subjected to nucleic acid extraction on **Trueprep® AUTO / AUTO v2** extraction system. (Refer to the User Manual of **Trueprep® AUTO / AUTO v2** Universal Cartridge Based Sample Prep Device and packinsert of **Trueprep® AUTO / AUTO v2** Universal Cartridge Based Sample prep kit. for details). ⚠ Dispose off the Transport Medium for Swab Specimen Tube, lysis buffer tube 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® Rabies** micro PCR chip, microtube and the DNase & RNase free pipette tip from the pouch, ensure that neither bare hands nor gloves that have been used for previous tests run are used.

13. PROCEDURAL LIMITATIONS

1. Optimal performance of this test requires appropriate specimen collection, handling, storage and transport to the test site.
2. Though very rare, mutations within the highly conserved regions of the target genome where the **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.

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 bio-hazard 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 "Rabies" 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 "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 chip-based Real Time PCR test and the microtube.
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 press the chip to ensure that it has seated in the chip tray properly.
9. Place the microtube containing freeze dried PCR reagents in the microtube stand provided along with the **Truelab®** Real Time micro PCR workstation **after ensuring that white pellet of dried PCR reagents remains at the bottom of the microtube.** Remove the microtube cap and dispose it off as per the section on "Disposal and Destruction" (Section 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® 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 18).
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 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® Rabies** 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®** Analyzer screen to indicate the progress of the test. Both the target and the internal positive control (IPC) curves will take a steep, exponential path when the fluorescence crosses the threshold value in case of positive samples. The time taken (Ct) of the specimen will depend on the number of target RNA in the sample. The curve will remain horizontal throughout the test duration and the IPC curve will take an exponential path in case of negative samples. In case the IPC curve remains horizontal in a negative sample, the test is considered as Invalid. At the end of the test run, the results screen will display "DETECTED" for Positive result or "NOT DETECTED" for Negative result. The result screen would also display the microbial 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 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.

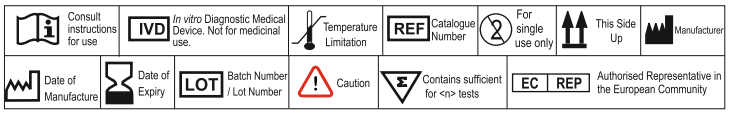
18. DISPOSAL AND DESTRUCTION

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.

19. REFERENCES

1. http://www.who.int/mediacentre/factsheets/fs099/en/1..
2. Sacramento, Debora, Herve Bourhy, and Noel Tordo (1991) PCR technique as an alternative method for diagnosis and molecular epidemiology of rabies virus.Molecular and cellular probes 5.3 : 229-240.
3. Crepin, P., et al. (1998) Intravitam diagnosis of human rabies by PCR using saliva and cerebrospinal Fluid. Journal of Clinical Microbiology36.4:1117-1121

SYMBOL KEYS



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