



# Evaluation of a rapid, chip-based, micro-PCR assay for detection of rabies virus in human and canine specimens

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## Abstract

Rabies, a lethal zoonotic encephalitis, remains a significant global health concern, causing an estimated 60 000 annual fatalities worldwide. Dogs serve as the primary reservoirs and vectors for transmitting this infection to humans. Definitive diagnosis of rabies in both human and animal cases necessitates laboratory testing involving various clinical specimens. However, the complexity of laboratory infrastructure and the need for skilled personnel, along with the challenge of maintaining cold-chain integrity during sample referral, hinder the decentralization of diagnostic facilities. This study aimed to assess the efficacy of the Truenat rabies assay, a rapid, portable, semiautomated, and closed PCR-based system, for the diagnosis of rabies in both humans and animals. The Truenat assay demonstrated a sensitivity of 100% and a specificity of 86.96% when compared with the fluorescent antibody test (FAT), as the reference standard, on 147 canine brain samples tested. Notably, the Truenat assay exhibited a sensitivity and specificity of 100% when tested on 48 human brain specimens. Furthermore, an examination of 148 human antemortem samples (cerebrospinal fluid, saliva, and skin biopsy) using both the Truenat assay and a validated real-time reverse transcriptase PCR assay revealed a  $\kappa$  value of 0.505, indicative of a moderate level of agreement between the two tests. Thus, the Truenat assay offers a robust, reliable, and affordable point-of-care solution to enhance rabies diagnostic capacity in endemic areas.

## KEYWORDS

molecular diagnostics, point-of-care test, rabies, rabies laboratory diagnosis, zoonoses

## 1 | INTRODUCTION

Rabies is a fatal zoonotic encephalitis that causes approximately 60 000 deaths annually across the globe. India accounts for about one-thirds (or approximately 20 000 fatalities) of the global toll.<sup>1</sup> Rabies is caused by the rabies virus (RABV) predominantly, and other viruses belonging to genus *Lyssavirus*, classified under Order *Mononegavirales*, and Family *Rhabdoviridae*. Human infections are commonly contracted through bites of rabid animals, primarily dogs.

A significant factor contributing to rabies being a neglected zoonosis is the absence of comprehensive disease surveillance in both humans and animals, resulting in a substantial underreporting of the true disease burden. To meet the global goal of “Zero human deaths due to dog-mediated rabies by 2030,”<sup>2</sup> recently the National Action Plan for Dog Mediated Rabies Elimination (NAPRE) by 2030, has been developed to prioritize rabies as a zoonosis and provide a strategic framework for control of rabies using an “One Health” approach in India.<sup>3</sup> Laboratory-based disease surveillance is a vital

component of this program, facilitating the assessment of disease prevalence, prompt identification and tracking of outbreaks, and the evaluation of intervention impact.

Due to the unreliable clinical signs of rabies in animals, a diagnosis of rabies can only be confirmed through detection of viral antigen in postmortem brain samples.<sup>4</sup> Swift confirmation in endemic areas allows for heightened efforts to identify all potentially exposed individuals, offer life-saving post-exposure prophylaxis (PEP) and reduce human rabies risk. Laboratory confirmation also informs control measures like mass dog vaccination by estimating disease prevalence in dogs and other animals.

Definitive diagnosis of human rabies requires testing of postmortem brain sample, often hindered by cultural, ethical, or logistical challenges. This lack of diagnostic opportunity exacerbates underreporting of human rabies. Enhancing antemortem diagnosis is vital for patient care and assessing actual rabies incidence. Antemortem confirmation involves detecting virus genome/antigen in samples such as saliva, nuchal skin and cerebrospinal fluid (CSF), and/or detection of antibodies in blood and CSF samples.<sup>1,5</sup>

However, these diagnostic assays necessitate a complex laboratory setup and skilled staff, hindering the decentralization of rabies diagnosis. Sample transportation to a rabies referral lab poses significant challenges due to cold-chain requirements, need for nucleic acid preservation, costs, and biosafety concerns. The availability of a point-of-care (POC) testing device which can be used on the field or at peripheral health centers can effectively address many of these challenges.

Truenat<sup>®</sup> Rabies (Molbio Diagnostics) is a chip-based real-time reverse transcription-polymerase chain reaction (RT-PCR) test for the semiquantitative detection of RABV in various clinical samples. This portable, battery-powered, semiautomated, closed PCR system is user-friendly and offers rapid results. This assay targets the nucleoprotein gene of RABV for amplification and has the capability to bring the advantages of molecular diagnostic methods to remote field locations.

Rabies is a classic One Health challenge, with human disease being driven by animal reservoirs, mainly dogs. Therefore, the aim of this study was to evaluate the Truenat rabies assay for its diagnostic performance using both human and canine specimens, and assess its utility as a POC test for diagnosis of rabies.

## 2 | MATERIALS AND METHODS

### 2.1 | Study design and clinical samples

A laboratory-based observational study was conducted in the Department of Neurovirology, a national rabies referral laboratory at the National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore, India over a period of 11 months.

Samples from suspected human and animal rabies cases, received in the laboratory for routine diagnostic confirmation from various parts of the country during the study period were included. Animal

samples included postmortem brain samples from dogs that had succumbed to suspected rabies, collected as a part of routine surveillance. Human specimens included antemortem specimens (saliva, CSF, and nuchal skin biopsy), and postmortem brain tissue specimens from suspected rabies cases, as well as previously characterized specimens from the Human Brain Tissue Repository (HBTR), Department of Neuropathology, NIMHANS, Bangalore. No additional human or animal sampling was performed specifically for this study. All human specimens used in this study were anonymized.

Ethical approval was obtained from the Institutional Ethics Committee [NIMH/DO/Ethics sub-committee (BS) meeting/2022" dated 10.12.2022 and "NIMH/DO/Ethics sub-committee (BS) meeting/2023" dated 17.07.2023].

### 2.2 | Fluorescent antibody test (FAT)

All human and animal brain tissues were subjected to FAT for detection of rabies nucleoprotein antigen, the gold standard for rabies diagnosis, as described earlier.<sup>6</sup> Briefly, smears were made from cut surfaces of fresh brain tissues, fixed in cold acetone for 2 h, air dried and treated with a cocktail of antirabies monoclonal antibodies conjugated with FITC (EMD Millipore Corporation) for 30 min at 37°C in an incubator in a humid chamber. Known rabies positive and negative brain smears were included as controls. The slides were examined under UV light using a fluorescent microscope.

### 2.3 | Real-time RT-PCR for detection of rabies RNA

Nucleic acid extraction from the clinical specimens was performed using commercially available extraction kit, that is, QIAamp Viral RNA minikit (Qiagen) as per manufacturer's instructions. Brain tissue and skin specimens were homogenized before the extraction procedure, while CSF and saliva specimens were used directly. One hundred and forty microliters of specimen was used and RNA was eluted in 60 µL of elution buffer. Real-time TaqMan RT-PCR using a set of primers and probe targeting the RABV nucleoprotein gene was performed on all the antemortem human clinical samples (CSF, saliva, and nuchal skin biopsy) as described previously.<sup>7,8</sup> A BIO-RAD C1000 Thermocycler instrument and software was used to perform PCR amplification and data analysis. All specimens were tested in duplicate and specimens with a mean threshold cycle ( $C_t$ ) value of  $\leq 35$  were considered positive.

The following controls were included, in every PCR run: positive control (RNA extracted from a known positive specimen); nontemplate control (template RNA replaced by nuclease free water); and extraction control (nuclease free water subjected to RNA extraction). Along with the RABV assay, a separate PCR reaction was set up for each human clinical specimen, for detection of RNase P (RP), a house-keeping gene target, to confirm adequacy of the specimen quality and the extraction procedure.

Results of the specimens which did not show the RP amplification were considered invalid.

## 2.4 | Truenat rabies assay

All human and canine samples included in the study were tested for presence of RABV RNA by the Truenat rabies assay. All brain tissue and nuchal skin specimens were homogenized, and other specimens were used directly for RNA extraction as per manufacturer's protocol. Five hundred microliters of specimen was used for the automated extraction procedure and 150 µL of eluted RNA was obtained (Figure 1).

*Left:* Trueprep<sup>®</sup> AUTO v2 Universal Cartridge Based Sample Prep Device for automated nucleic acid extraction in 20 min using a sample processing cartridge; *Right:* Truelab<sup>®</sup> Duo Real Time Quantitative micro PCR Analyzer, which is a portable micro real-time quantitative PCR system with data connectivity, 8 h battery backup and 3 channel fluorescence. Devices with different throughputs are also available.

Following the manufacturer's instructions, RNA extraction was performed using Trueprep<sup>®</sup> AUTO v2 Universal Cartridge Based Sample Prep Device and corresponding kit. This was followed by PCR amplification and analysis on the Truelab<sup>®</sup> Duo Real Time Quantitative micro-PCR Analyzer. At the end of the test run, the results were displayed on the screen as "DETECTED" for Positive result or "NOT DETECTED" for Negative result. The result screen (Figure 2) also displayed the validity of the test run as "VALID" or "INVALID." For positive specimens, the result screen displayed the viral load as "HIGH" ( $C_t < 20$ ), "MEDIUM" ( $20 \leq C_t < 25$ ), "LOW" ( $25 \leq C_t < 30$ ), or "VERY LOW" ( $C_t \geq 30$ ).

Figure shows display of result for a sample negative for rabies viral RNA (left), and a sample positive for rabies viral RNA (right).

Each Truenat Rabies PCR reaction includes an internal positive control (IPC) to determine the validity of the test run. The IPC is a 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  $C_t$  beyond a pre-set range in case of negative samples invalidated the test run. In positive samples, especially those with high viral RNA load, the IPC may not amplify, however, these results are to be considered positive.

## 2.5 | Statistical analysis

For canine and human brain specimens, the results of Truenat rabies assay were compared with the reference gold standard test, that is, the FAT. Estimates of diagnostic accuracy and their precision were calculated using GraphPad and MedCalc online tools. A separate analysis was carried out to compare the performance of Truenat assay with routine RT-PCR for antemortem human specimens. The degree of agreement between the two tests was determined using Cohen's  $\kappa$  measurement.

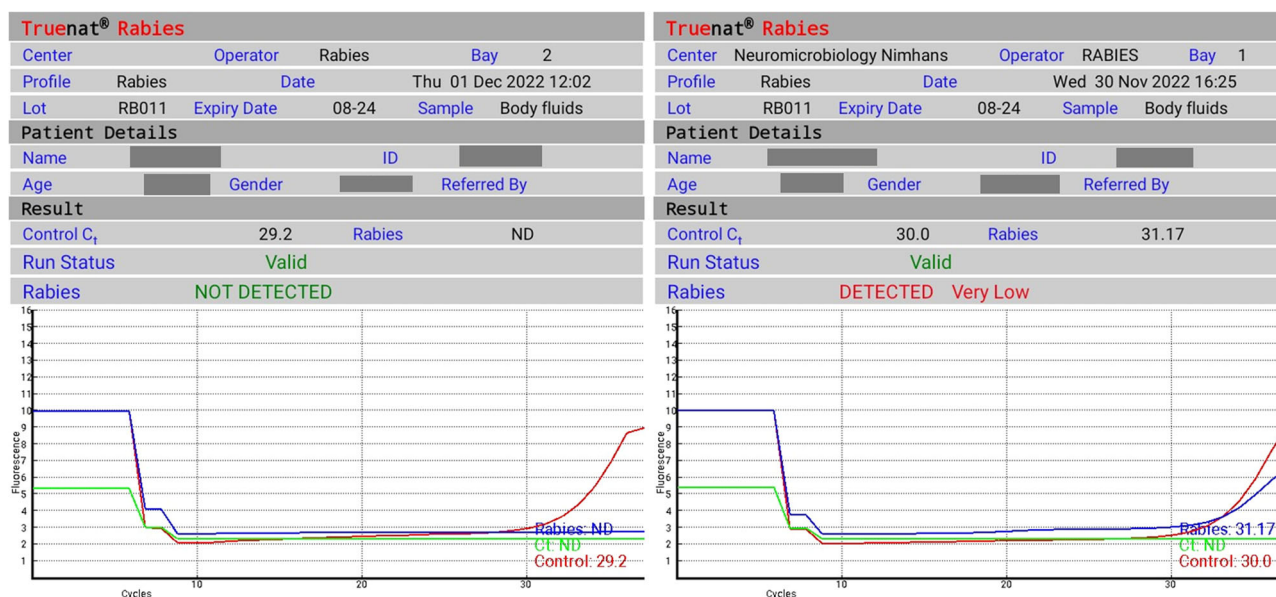
## 3 | RESULTS

### 3.1 | Diagnostic performance of Truenat rabies assay: Dog brain samples

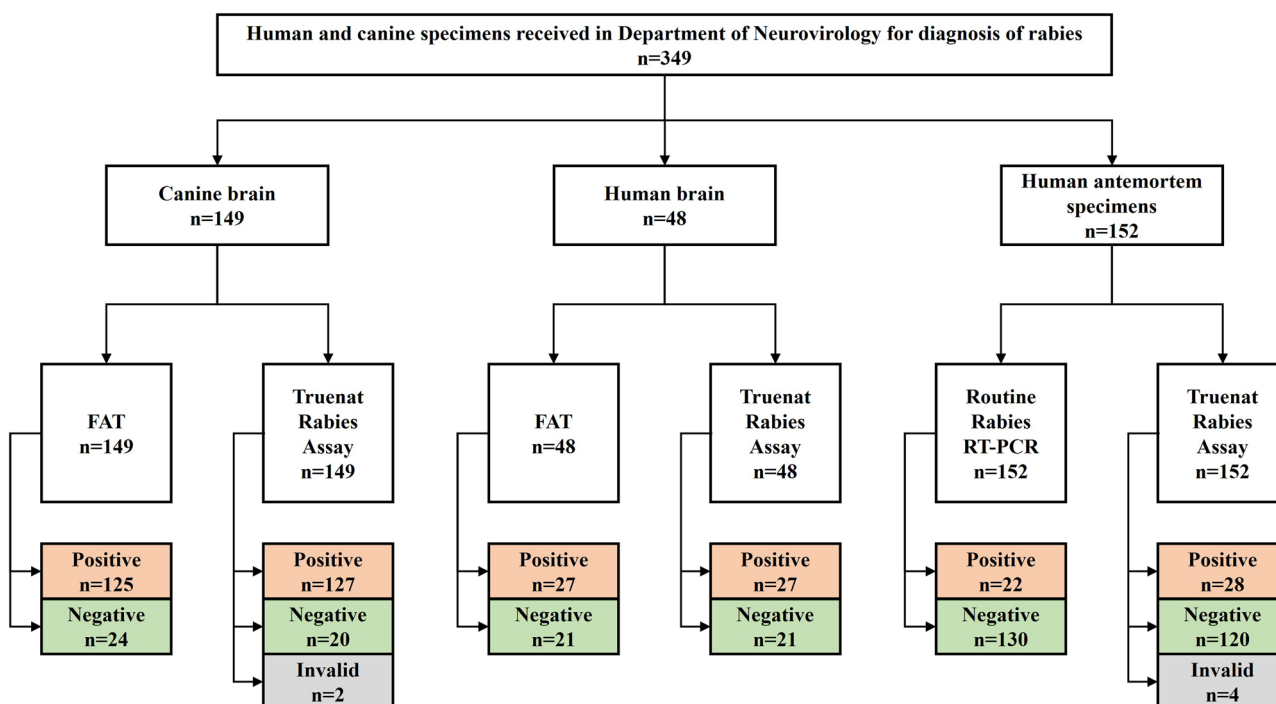
A total of 149 dog brain specimens were tested with both the Truenat assay and FAT (Figure 3 and Table 1). Eight specimens (5.37%) were initially reported invalid in the Truenat assay, and in six of these, a valid result was obtained by either repeating the PCR step from the original eluted RNA (three specimens), or by repeating both extraction and PCR from the specimens (three specimens). Among



FIGURE 1 Truenat instrumentation.



**FIGURE 2** The result display of Truenat rabies assay.



**FIGURE 3** Results of diagnostic tests performed on various clinical samples. FAT, fluorescent antibody assay.

the two unresolved specimens, one was reported positive by FAT, and repeat extraction for Truenat could not be performed due to paucity of material. The other unresolved specimen had tested negative with FAT, and Truenat result remained invalid even after repeating extraction and PCR. These two invalid specimens were excluded from the statistical analysis for the calculation of diagnostic performance indicators.

The Truenat results of 147 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%).  $\kappa$  was calculated to be 0.918, with standard error of 0.047 and 95% CI of 0.82720–1.00000, signifying very good agreement between the two tests. The two-tailed  $p$  value

**TABLE 1** Results of FAT and Truenat rabies assay for canine brain specimens.

Fluorescent antibody test (FAT) result (n = 149)	Truenat rabies result	Number (Percentage of FAT results)
Negative (n = 24)	Detected	3 (12.50%)
	Not detected	20 (83.33%)
	Invalid	1 (4.17%)
Positive (n = 125)	Detected	124 (99.20%)
	Invalid	1 (0.80%)

**TABLE 2** Results of FAT and Truenat rabies assay for human brain specimens.

Fluorescent antibody test (FAT) result (n = 48)	Truenat rabies result	Number (Percentage of FAT results)
Negative (n = 21)	Not detected	21 (100%)
Positive (n = 27)	Detected	27 (100%)

calculated by Fisher's exact test was less than 0.0001, thus these findings are statistically significant.

### 3.2 | Diagnostic performance of Truenat rabies assay: Human brain samples

A total of 48 human postmortem brain specimens were tested with both the Truenat assay and the gold standard test, that is, FAT. All specimens yielded valid Truenat results, and on comparison with the reference standard, the sensitivity of Truenat was found to be 100% (95% CI 87.23%–100%), and the specificity was 100% (95% CI 83.89%–100%).  $\kappa$  was 1.00, signifying perfect agreement between the tests (Figure 3 and Table 2).

### 3.3 | Diagnostic performance of Truenat rabies assay: Human antemortem samples

A total of 152 human antemortem specimens were tested with the Truenat assay and the routine RT-PCR, which included CSF (n = 77, 50.66%), saliva (n = 56, 36.84%), and skin (n = 19, 12.50%) specimens. Initially, 11 (7.24%) specimens, that is, 6 CSF and 5 saliva specimens, tested as invalid with the Truenat assay, out of which 7 were resolved by either repeating the PCR (in 1 CSF and 1 saliva specimen) or by repeating the PCR with a freshly extracted elute (in 1 CSF and 4 saliva specimens). The remaining four CSF specimens yielded invalid results even after repeating Truenat extraction and PCR steps. The results of both assays are summarized in Table 3 and the detection of rabies viral RNA from various human specimens is depicted in Figure 4.

**TABLE 3** Results of routine RT-PCR and Truenat rabies assay for human antemortem specimens.

Routine rabies RT-PCR result (n = 152)	Truenat rabies result	Number (Percentage of Routine rabies RT-PCR result)
Positive (n = 22)	Detected	14 (63.64%)
	Not detected	6 (27.27%)
	Invalid	2 (9.09%)
Negative (n = 130)	Detected	14 (10.77%)
	Not detected	114 (87.69%)
	Invalid	2 (1.54%)

Thus, valid Truenat results were obtained for 148 antemortem specimens. Out of these, Truenat result was concordant with the RT-PCR for 128 (86.49%) specimens, and discordant in 20 (13.51%). Among the discordant specimens, there were 6 specimens (2 each of CSF, saliva, and skin) that were RT-PCR positive but Truenat negative, and 14 specimens (3 CSF, 7 saliva, and 4 skin) that were RT-PCR negative but Truenat positive.

Statistical analysis was performed for 148 specimens (after excluding the specimens invalid by Truenat assay), and  $\kappa$  was calculated to be 0.505 with standard error of 0.095 and 95% CI of 0.319–0.691, signifying moderate agreement. For CSF specimens, routine RT-PCR positivity rate was higher than Truenat rabies assay (12.99% vs. 11.69%). For saliva and skin specimens, Truenat rabies positivity rate was higher than routine RT-PCR (23.21% vs. 14.29% for saliva and 31.58% vs. 21.05% for skin). Overall, Truenat rabies positivity rate was higher than routine RT-PCR for all human antemortem specimens combined (18.42% vs. 14.47%).

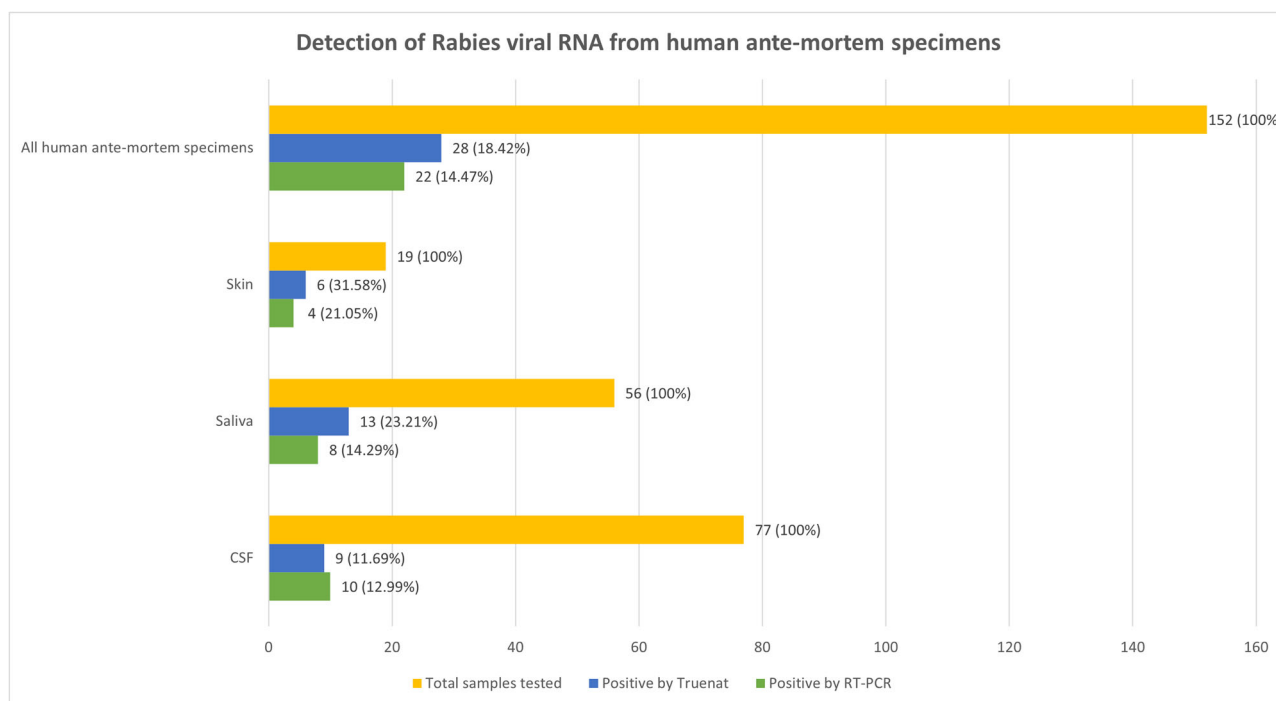
There were a few technical challenges during the Truenat testing with human specimens. First, there were several instances where the RNA extraction cartridges were “clogged” due to mucoid specimens and the extraction procedure failed. This was resolved by the use of a liquefaction buffer for such specimens. There were also two instances of technical errors (Error codes 1 and 3) during the PCR procedure, both were resolved by repeating the PCR step with a new chip.

## 4 | DISCUSSION

India has a substantial rabies burden, contributing significantly to global mortality. Yet, underreporting persists due to the lack of laboratory confirmation. Laboratory confirmation of rabies is pivotal for accurate reporting, enabling proper care in affected humans, supporting disease surveillance and implementation of targeted prevention and control measures.

Viral antigen detection by FAT on brain tissue obtained postmortem, is the current gold standard for detection of RABV in both animals and humans.<sup>5</sup> However, this test requires an expensive fluorescent microscope and expertise in interpretation of results.





**FIGURE 4** Detection of rabies viral RNA from human antemortem specimens.

Moreover, several fields in the smears have to be screened, especially to confirm a negative result, which is time-consuming. Furthermore, maintaining a cold chain during sample shipment to the laboratory proves particularly challenging, especially in tropical and subtropical nations, and significantly impedes the effective utilization of this test.

Lateral flow devices or immunochromatographic assays for rapid detection of RABV antigens in animal brain specimens under field conditions are available. However, while some studies have reported results comparable to FAT,<sup>9,10</sup> a lack of standardization and quality control of kits has been reported by others.<sup>11</sup> Moreover, these tests serve as initial screening tools and necessitate confirmation through FAT or other assays.

FAT is not suitable for antemortem diagnosis in clinically suspected human rabies cases, a critical step in distinguishing from clinical mimics, managing patients, and initiating timely PEP for close contacts. Currently, antemortem diagnosis is done by detection of viral RNA in various clinical samples and/or antibody detection in CSF and blood samples. Widespread field utility of these assays is restricted by requirement of extensive laboratory setup and skilled personnel. Moreover, the necessity for multiple or serial sample testing to enhance diagnostic sensitivity, coupled with the challenge of maintaining a cold-chain during sample transportation to the laboratory, presents significant barriers in resource-limited settings.<sup>12,13</sup>

Thus, the lack of a POC diagnostic test for use in suspected human and animal cases is a major challenge for control programs.<sup>14</sup> There is a need for an easy, rapid, and cost-effective POC antemortem and postmortem diagnostic test to confirm human rabies in primary health care centers or peripheral labs, as well as a

POC postmortem diagnostic test for use in animals under field conditions. To this end, the Truenat rabies assay is a promising field-deployable alternative to the currently available diagnostic techniques, that can aid in the decentralization of laboratory facilities, and enhancing surveillance in the most underserved regions.

The Truenat platform has already been approved by the World Health Organization (WHO) for diagnosis of tuberculosis,<sup>15</sup> and is also being widely used for diagnosis of other infectious diseases such as COVID-19.<sup>16</sup> The Truenat rabies assay has been evaluated in a small pilot study for animal brain specimens,<sup>17</sup> and is approved for use on animal specimens. It is currently not validated for detection of RABV from human ante- and postmortem specimens.

This study is important not only because of the substantial sample size, but also because of the wide range of specimens studied. Since this laboratory is a national referral laboratory for rabies, the sources of the specimens included in this study are truly representative of the spectrum of rabies incidence in the country. The human specimens were received from all over the country from private as well as public sector healthcare institutions of different capacities and levels of diagnostic capabilities. The animal specimens were received from field surveillance operations in different parts of the country, as well as from veterinary healthcare institutions.

#### 4.1 | Diagnostic performance of Truenat on canine and human brain specimens

The performance of Truenat rabies assay for animal brain specimens has only been evaluated in one study so far, comprising of 24 animal

specimens in Kerala state, India. The sensitivity, specificity, and diagnostic accuracy of Truenat rabies assay compared with FAT was 92.3%, 100%, and 95.8%, respectively.<sup>17</sup> In the current study, which included a much larger number ( $n = 149$ ) of canine brain specimens, the sensitivity, specificity, and diagnostic accuracy of Truenat was found to be 100%, 86.96%, and 97.96% respectively. The lower specificity in the current study is due to the three specimens that were FAT-negative, but positive by Truenat. Although these are labeled as “false positive,” they are likely to be true positives, because of enhanced sensitivity of Truenat assay. It has already been demonstrated that molecular methods are able to detect viral RNA in suboptimal or decomposed specimens where FAT fails to detect antigens.<sup>18–21</sup>

Two canine brain specimens yielded invalid results with the Truenat assay. While these unresolved cases underscore a limitation of the Truenat assay, it's important to recognize that such challenges are an expected aspect of the developmental journey for any novel diagnostic technique. During a feasibility assessment for Truenat tuberculosis assay, it was seen that the number of invalid results declined over time, presumably due to the increased familiarity and efficiency of the operators.<sup>22</sup> Furthermore, the presence of unknown PCR inhibitors in the specimens cannot be ruled out, as many such inhibitors have been described to interfere with the PCR reaction at different steps.<sup>23</sup> Nevertheless, these outcomes signal the necessity for additional troubleshooting and refinement of the assay, encompassing both the extraction and PCR phases.

This study is the first to evaluate the Truenat rabies assay on human brain specimens ( $n = 48$ ), and the assay demonstrated a sensitivity and specificity of 100%. The excellent diagnostic performance of Truenat assay for human and animal brain specimens makes it a suitable alternative for FAT at field level as well as in established laboratories.

## 4.2 | Performance of Truenat on human antemortem specimens

The Truenat assay demonstrated a higher positivity rate than RT-PCR with saliva and skin biopsy samples, but not with CSF. Among all the 152 specimens tested, 6 RT-PCR positive specimens (2 CSF, 2 saliva, and 2 skin biopsy) were negative by Truenat, and 2 RT-PCR positive CSF specimens were invalid by Truenat. This indicates the scope for further optimization of the assay and the specimen processing methods.

On the other hand, 14 RT-PCR negative specimens (7 saliva, 4 skin biopsy, and 3 CSF) tested positive by Truenat. Since the human specimens were all obtained from clinically suspected rabies cases, these are likely to be true positives. Furthermore, in two of these cases, rabies viral RNA was detected by both RT-PCR and Truenat in a different clinical specimen from the same patient, thus supporting the positive results obtained by Truenat.

Another mobile PCR device which has been evaluated in the Philippines for diagnosis of rabies is the PCR 1100 assay which

utilizes the pan-lyssavirus LN34 primer system. The sensitivity and specificity of this assay was reported to be 100% when tested with canine and feline brain specimens obtained from the field.<sup>24</sup> Although the PCR 1100 system is a battery powered mobile device, RNA extraction has to be performed in a laboratory using a separate commercial kit and related equipment. Thus, compared with Truenat assay, this technique may have limited field utility.

## 4.3 | Practical application and implementation of Truenat rabies assay in high-burden settings

To address the existing gap in decentralized rabies diagnostics within India and other rabies endemic countries, the Truenat rabies assay emerges as a fitting and practical solution for deployment at peripheral laboratories. The Truenat platform is already widely used for diagnosis of tuberculosis,<sup>22,25,26</sup> COVID-19,<sup>16</sup> and other infectious diseases.<sup>27,28</sup> Hence rabies testing can be seamlessly integrated into the pre-existing diagnostic services with minimal additional expenditure on infrastructure or training of human resources. The initial cost of setting up the Truenat platform is approximately USD 6000–8000, and the subsequent cost per test is approximately USD 10.

The advantages of this portable, battery-operated, IoT-enabled, POC real-time PCR platform include the ability to function without a constant power supply, minimal need for complex lab infrastructure, and user-friendly operation with a rapid turnaround time of about 1 h with GPRS/Bluetooth enabled data transfer. The assay processes samples individually, mitigating cross-contamination risks. Furthermore, its reagents remain stable at temperatures up to 40°C, eliminating the need for refrigeration. Availability of this platform can eliminate the need for sample storage and transportation from peripheral areas and reduce the burden of testing at referral centers. It can provide rapid results in the field and serve as a convenient POC test during outbreaks.

One of the limitations of Truenat rabies assay is the lack of a sample adequacy or RNA quality check, similar to the detection of a human housekeeping gene in real-time PCR assays. Although there is an in-built internal control, it only serves the purpose of confirming that the extraction and amplification steps have been performed correctly. However, in case of a valid negative result, it is not possible to ascertain whether the specimen quality is optimal. Nonetheless, optimizing the internal control could pose a challenge due to the shared utilization of the system for both animal and human clinical samples.

Also, in case of an invalid result, it is not possible to pinpoint the reason which may include errors in RNA extraction, or amplification, or the presence of PCR inhibitors in the specimens. It may be necessary to repeat amplification step with the same elute, or repeat both steps from the specimen, or test the sample using a different test to get a valid positive or negative result. Re-extraction from the specimen may not always be feasible because of scarcity of specimens and the difficulty in obtaining a repeat specimen. Further

optimization of the assay may be able to eliminate the issue of invalid results and also reduce the sample volumes required for testing.

## 5 | CONCLUSION

The Truenat assay is a robust, reliable, and affordable POC test for diagnosing rabies in both humans and animals. Coupled with optimized sample collection techniques, the Truenat platform can strengthen the One Health approach to dog-mediated rabies control by boosting diagnostic capabilities across all healthcare levels in rabies endemic countries.

## AUTHOR CONTRIBUTIONS

**Lonika Lodha:** Investigation; data curation; visualization; writing—original draft preparation. **Ashwini M. Ananda:** Methodology; project administration; resources; writing—review and editing. **Arya Ramachandran:** Data curation; investigation; project administration. **Sathya Priya Manuel:** Investigation; validation. **Sujatha Valagere Sannaiah:** Investigation; validation. **Anita Mahadevan:** Investigation; resources. **Reeta S. Mani:** Conceptualization; methodology; resources; supervision; writing—review and editing.

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**How to cite this article:** Lodha L, Ananda AM, Ramachandran A, et al. Evaluation of a rapid, chip-based, micro-PCR assay for detection of rabies virus in human and canine specimens. *J Med Virol*. 2023;95:e29110. doi:10.1002/jmv.29110