

Comparative Assessment of the Rapid Immune Chromatographic Test (RICT) and Direct Fluorescent Antibody Test (DFAT) for Rapid and Accurate Laboratory Diagnosis of Rabies.

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Abstract- Rabies is a fatal zoonotic disease that continues to present a public health challenge globally, in particular the endemic regions such as sub-Saharan Africa. In Nigeria, rabies is mainly acquired through dog-mediated exposures, and indeed the true burden is often underestimated due to poor surveillance and diagnostic tools. Rapid and accurate laboratory diagnosis is vital to effective post exposure prophylaxis (PEP) and outbreak control. The Direct Fluorescent Antibody Test (DFAT), is considered to be the gold standard and is reliable for detecting rabies, but it requires proper trained personnel and specialized equipment which is needed in many resource-limited areas. This investigation examined how good a Rapid Rabies Test (RRT), a type of Rapid Immune Chromatographic Test (RICT), is by comparing it to the DFAT on a cohort of 234 canine brain tissues from Nigeria's North Central states (Benue, Nasarawa, and Plateau). The DFAT found a prevalence of Rabies virus (RAV) antigens of 8.12% in the cohort examined. The RRT diagnostic test was found to have a sensitivity of 78.9% (95% CI: 56.7–92.2%), and specificity of 98.1% (95% CI: 95.7–99.4%). The high specificity of the RRT diagnostic test supports its use as a quick and on-site screening mechanism for rabies and as an early detection test, while the moderate sensitivity indicates that some cases may be missed, resulting in a false negative test result. This report concludes that RRT is a great preliminary screening tool for rabies in the field, and is certainly an acceptable test for rabies screening, it should not be used as a stand-alone diagnostic test and not replace the DFAT that would be used to ultimately assess canine rabies, especially if it is to be used as part of public health interventions, as the public health consequences of missing rabies cases could be quite acute.

Keywords: Rabies, Diagnosis, Rapid Rabies Test, Direct Fluorescent Antibody Test, Sensitivity, Specificity, One Health

I. INTRODUCTION

Rabies is a viral zoonosis that leads to severe encephalitis in mammals and is nearly always fatal once clinical neurological symptoms manifest. Humans die from rabies at high rates around the world, with more than 59,000 deaths attributed to rabies each year, primarily in parts of Asia and Africa (WHO, 2016). In Nigeria, the combination of a large, unvaccinated population of domestic dogs and common cultural practices associating very close distance between humans and animals, leads to one of the highest rabies burdens in Africa (Mshelbwala *et al.*, 2021). Dog rabies burden results from a fractured public health system, as well as the ongoing shortage of human and animal medicine, which limits rabies outbreak surveillance and a response (Adedeji *et al.*, 2010).

To ensure effective rabies control and prevention, rapid and accurate laboratory diagnosis of suspected cases is essential. The Direct Fluorescent Antibody Test (DFAT) is endorsed as the gold standard rabies diagnostic test by both the World Health Organization (WHO) and the World Organisation for Animal Health (OIE), due to the sensitivity and specificity of the test. The DFAT measures the rabies virus nucleoprotein from brain tissue, using a fluorescently labeled antibody for detection. However, the DFAT is a laborious laboratory diagnostic method that requires specialized equipment, such as a fluorescent microscope, dedicated laboratory space, and trained laboratory staff (WHO, 2005). Unfortunately, these criteria are often impractical in rural and peri-urban parts of

Nigeria where rabies is commonly diagnosed, thus creating a major diagnostic gap resulting in underreporting of rabies cases and delayed access to life-saving post-exposure prophylaxis (PEP) (Abubakar *et al.*, 2023).

In the interest of responding to this gap, there have been a number of alternative diagnostic strategies developed, most notably Rapid Diagnostic Tests (RDTs). RDTs are quicker, more portable, and technically less demanding to perform compared to the tests previously mentioned, making them more suitable for use in the field and/or in point-of-care (Alvarado-Fernández *et al.*, 2023). The Rapid Immune Chromatographic Test (RICT), also known as a rapid rabies test (RRT), is one type of RDT that is thought to provide a valuable option for preliminary screening. Similar to Lateral Flow Assays, RICTs identify viral antigens visually and have the potential for providing results in a timespan of only a few minutes. The World Health Organization (WHO), has acknowledged the potential of rapid tests to increase rabies surveillance in locations with poor access to laboratory diagnostics (WHO, 2016).

While the potential benefits of RICT are promising, there is very little data that considers its performance in Nigeria. Previous studies in Nigeria have focused on prevalence, with limited reports that compare the accuracy of any diagnostic methods. This gap in knowledge has prohibited the implementation of a tiered and efficient diagnostic approach. For this purpose, this study is being conducted to evaluate a Rapid Rabies Test (RRT) by evaluating its sensitivity and specificity compared to the gold standard, DFAT, with a large sample of canine brains from rabies endemic region in North Central Nigeria. The outcomes will provide important baseline data for future considerations in using rapid tests to develop a more comprehensive rabies endemic surveillance system to increase rabies diagnostic capabilities in Nigeria.

II. METHODOLOGY

Study Area and Sample Population

The research was carried out on a total of 234 canine brain specimens collected from designated slaughterhouses and abattoirs in the North Central geopolitical zone of Nigeria. The samples were collected from three major states: Benue, Nasarawa, and Plateau, which were purposely selected due to the presence of a relatively high population of canine animals, known trade in dog-meat, and a reported history of human rabies cases. The period of study took place during 2022 to 2023 to provide representative sample collection over a period of time.

Sample Collection and Handling

Brain tissue samples were obtained from the medulla oblongata, the base of the cerebellum, and the hippocampus of dogs slaughtered for human consumption. This selection was based on the premise that the rabies virus is most concentrated in these neural tissues (WHO, 2005). To minimize the risk of cross-contamination and sample degradation, each sample was aseptically obtained using a different sterile surgical blade and placed into sterile labeled vials. The samples were immediately stored on-site at -20°C and were transported with strict cold chain management to the National Veterinary Research Institute (NVRI) located in Jos, Nigeria, where they were stored at -80°C until laboratory analysis.

Laboratory Diagnosis: The Reference Standard (DFAT)

All 234 brain samples were subjected to the Direct Fluorescent Antibody Test (DFAT) following protocols from the OIE and WHO (WHO, 2005; OIE, 2004a). Impression smears of brain tissue were made on clean glass slides, air-dried, fixed in cold acetone at -20°C for 30 minutes to inactivate the virus, and then stained with a commercial FITC-conjugated anti-rabies monoclonal antibody (Bio-Rad). After 30 minutes of incubation at 37°C in a humid chamber, the slides were thoroughly washed with phosphate buffered saline (PBS) to remove unbound conjugate. A mounting medium was applied before the slides

were examined under a fluorescent microscope (Olympus BX53) at 200 \times magnification. Positive test results indicated distinct, apple-green fluorescent viral inclusions (Negri bodies) in neuronal cells. Positive (rabid mouse brain) and negative (rabies-free dog brain) controls were included for quality control in each batch.

Laboratory Diagnosis: Test Method (RICT/RRT)

In addition to the DFAT, a commercially available Rapid Rabies Test (RRT) was also performed on each brain sample using the Bionote Inc. Anigen Rapid Rabies Ag Test Kit. This RRT is a lateral flow immunochromatographic assay that qualitatively detects rabies virus nucleoprotein antigens. A small piece of brain tissue was crushed in the supplied assay diluent, creating a suspension. After mixing according to the manufacturer's instructions, a few drops of the suspension were added to the sample well on the test cassette, and after 10 to 20 minutes, reading of the results occurred. Both control line (C) present and test line (T) present indicates a positive result, whereas only the control line indicates a negative result.

Statistical Analysis

The data from both assessments were entered into a database for the purpose of analysis. The RRT's diagnostic performance was evaluated using DFAT as the reference standard. The following metrics and 95% Confidence Intervals (CI) were calculated:

- Sensitivity is the percentage of DFAT positive samples correctly identified as positive samples by RRT.
- Specificity is the proportion of DFAT negative samples correctly identified as negative samples by RRT.

Statistical analyses were conducted using Microsoft Excel and SPSS version 25.

IV. RESULTS

A total of 234 canine brain tissue samples were analyzed in this study. DFAT, which is considered the gold standard, identified 19 samples that were positive for rabies virus antigens, which resulted in

an overall prevalence of 8.12% (95% CI: 4.88–12.56%).

The performance of the Rapid Rabies Test (RRT) was measured against the DFAT result as follows:

- The RRT accurately identified 15 of the 19 DFAT positive samples as positive samples.
- The RRT accurately identified 211 of the 215 DFAT negative samples as negative samples.

Based on the preceding outcome, Table 1 depicts the metrics of diagnostic performance for the Rapid Rabies Test (RRT).

Table 1: Comparative Diagnostic Performance of the Rapid Rabies Test (RRT) Versus the Direct Fluorescent Antibody Test (DFAT)

Diagnostic Parameter	Value (%)	95% Confidence Interval
Sensitivity	78.9%	56.7% - 92.2%
Specificity	98.1%	95.7% - 99.4%
Positive Predictive Value (PPV)	88.2%	63.6% - 98.5%
Negative Predictive Value (NPV)	96.3%	93.3% - 98.1%

The sensitivity of the RRT was 78.9%, indicating it detected just under four out of five true positives. The specificity was high at 98.1%, with few false positives.

V. DISCUSSION

This comparative evaluation provides pivotal evidence that supports the practicality of rapid diagnostic methods for rabies surveillance and control in Nigeria. The overall rabies prevalence of 8.12% (95% CI: 4.88–12.56%) estimated using a gold standard Direct Fluorescent Antibody Test (DFAT) in this set of slaughtered dogs (Okoh *et al.*, 2018) provides confirmation of the continued endemic status of rabies in the North Central Nigeria region (Mshelbwala *et al.*, 2021; Konzing *et al.*, 2023). However, it is the core value of this study to assess how the use of a Rapid Immune

Chromatographic Test (RICT), referred to here as the Rapid Rabies Test (RRT), can address the diagnostic gap between DFAT's logistical complications and the resource commitment when diagnosing rabies in rural and peri-urban/sub-urban Nigeria (WHO, 2005; Abubakar *et al.*, 2023).

High specificity of the Rabies Rapid Test (RRT), which achieved a specificity of 98.1%, is an auspicious finding that could have strong implications for public health. A positive RRT test result is very trustworthy and can serve as an excellent first-line screening test for a disease where such action is warranted (Okoh *et al.*, 2018). High levels of specificity are of particular interest in settings limited by resources in that it reduces falsely positive results that may result in misallocation of scarce resources, such as the unnecessary provision of expensive, if not scarce, Post-exposure Prophylaxis (PEP) to individuals that perhaps were never in contact with the virus (WHO, 2005; WHO Rabies Modelling Consortium, 2020). This type of reliability for frontline public health workers and veterinarians is necessary to feel confident in their tests so they can respond quickly, such as quarantining an animal or advising immediate wound care until definitive laboratory testing is done (Kayali *et al.*, 2023).

On the contrary, the moderate test sensitivity of 78.9% raises a serious issue. The RRT missed rabies virus antigens in 4 of the 19 samples that tested DFAT-positive, which means there is some risk for false negatives. Rabies approximately has a 100% case fatality rate, so potentially missing this diagnosis can have disastrous consequences. Failure to provide rabies post-exposure prophylaxis (PEP) for a rabies exposure event may potentially be fatal (Rupprecht *et al.*, 2018). This result is consistent with similar research in various other developing countries with some rapid tests proving useful but not entirely reliant as a diagnostic method (Alvarado-Fernández *et al.*, 2023). In another study in Costa Rica, for instance, sensitivity was less than ideal, further contributing to the worldwide concern about dependence of RDTs (Alvarado-Fernández *et al.*, 2023). This finding is an important warning - while RRTs enhance access, they need to be adapted within

a system that minimizes their diagnostic shortcomings.

Although the DFAT-confirmed prevalence of 8.12% among slaughtered dogs is a conservative statistic, it nevertheless strongly illustrates the endemic cycle of the virus in North Central Nigeria. Prevalence in a sentinel population (slaughtered dogs) in an endemic setting such as this is an important and actionable measure of community-level risk, in addition to passive surveillance data (Hampson *et al.*, 2015; Mshelbwala *et al.*, 2021).

The diagnostic performance of the RRT is informative for how we interpret reported prevalence. If the RRT were used as the sole diagnostic tool and if the sensitivity of 78.9% were maintained, then for every five rabies cases (approximately) that test as positive, we would expect to miss and mark negative, an additional positive rabies case. These systematic under-detections would artificially depress prevalence and risk being under-reported as policymakers may justify response actions in relation to the false prevalence or disease burden shown by the under-detected prevalence and tarred by the reported prevalence being lower than it would be with the DFAT method (Abubakar *et al.*, 2023; Kuye *et al.*, 2024). This emphasizes the importance of the DFAT—and alternative molecular methods with high sensitivity—as a gold standard for epidemiological tracking.

The performance metrics strongly support the requirement of “a tiered diagnostic approach,” that, will contribute to rabies control strategies in Nigeria (Abubakar *et al.*, 2023). This model requires a specific movement of samples and information:

Field Triage (RICT/RRT): The RRT can be quickly implemented at rural veterinary extension offices, abattoirs, and health centers, providing a fast, preliminary result in minutes (World Health Organization, 2016). All RRT positive cases can be tagged immediately for a public health response, such as notifying exposed persons and commencing PEP, minimizing the response time (O'Donnell *et al.*, 2019).

Confirmatory Testing (DFAT/PCR): All specimens from suspected rabies exposures (regardless of the result of the RRT), especially RRT-negatives after a high-risk exposure, must be submitted immediately, without hesitation, to a centrally located, well-resourced laboratory for DFAT or RT-qPCR confirmatory testing (WHO 2005; Awoyomi *et al.* 2019). This two-part approach affords rapid action while preserving the high diagnostic standards needed for appropriate case management, as well as reliable epidemiological information. Thus, the RRT, while an essential triage diagnostic that decreases the time to suspicion, ultimately does not replace the DFAT as the definitive diagnostic triage standard for case confirmation and epidemiological submission (Okoh *et al.* 2018).

Integrating the RRT within the framework of national surveillance is a rational investment based on its cost-effectiveness to the public health system. While DFAT will require capital outlay in fluorescent microscopes, cold chain logistics for specimen transport, and staff trained in rabies diagnosis, RRT is low-cost per test, low-training/skill, and portable. The RRT is an ideal tool for ruling out rabies in high-volume, low-risk specimens due to its good Negative Predictive Value, allowing reference laboratories to appropriately focus DFAT resources on the true complex or higher risk cases (WHO Rabies Modelling Consortium 2020).

This is in strong support of the One Health approach (Blumberg 2024). Enabling frontline workers access to RRT is a significant enhancement to data capture and the response time (Kuye *et al.* 2024). Allowing for quick decisions in the field and appropriately directing any confirmatory testing will provide a tier system that bridges human and animal health sectors and makes rabies control programme more efficient, scalable, and ultimately, more successful towards the global goal of dog-mediated human rabies elimination by 2030 (Abubakar *et al.* 2023; WHO 2023).

VI. CONCLUSION

This comparative study furthers the evaluation of the diagnostic capacity of the Rapid Rabies Test (RRT), a Rapid Immune Chromatographic Test (RICT), in

comparison to the gold-standard Direct Fluorescent Antibody Test (DFAT), as it used 234 canine brain samples collected from the endemic North Central region of Nigeria. The DFAT gold standard confirmed rabies prevalence in slaughtered dogs at 8.12%, implying the regional rabies endemic cycle continues and is a public health risk. The RRT had an excellent specificity of 98.1% (95% CI: 95.7–99.4%) establishing it as a good initial screening test for rules in rabies cases at point-of-care in the field, such as an abattoir or remote clinic. High specificity helps direct limited resources, such as Post-Exposure Prophylaxis (PEP), to immediate public health needs, as well as providing swift initial public health interventions. However, the moderate sensitivity of the RRT (78.9%, 95% CI: 56.7–92.2%) is also an important limitation, as the RRT missed 1 in every 5 true positive cases, creating a serious public health threat due to rabies having almost 100% fatality rate.

Consistent with the objective of closing the diagnostic gap in under-resourced settings while maintaining diagnostic robustness, we conclude that the RRT should not supplant the DFAT as the final diagnostic gold standard for rabies. Rather, we feel that it is best positioned as a component of a diagnostic hierarchy, where it can act as an initial rapid field triage, with all samples flagged as suspicious (particularly high-risk RRT-negative samples) requiring onward referral to DFAT or another highly sensitive molecular platform (i.e., RT-qPCR) for definitive diagnosis. This approach is the most expedient and responsible mechanism for furthering rabies surveillance in Nigeria and towards the goal of global elimination.

Recommendations

Upon review of our findings, and recognizing the need for a more tiered diagnostic approach, prior to the eventual need of optimizing rabies control in Nigeria would be to define the following research priorities:

Field Validation of the Tiered Diagnostic Approach: Conduct a prospective implementation study to pilot the proposed tiered diagnostic pathway (Field RRT → Central DFAT/RT-qPCR) in three North Central Nigerian States. The study should evaluate: (1) the

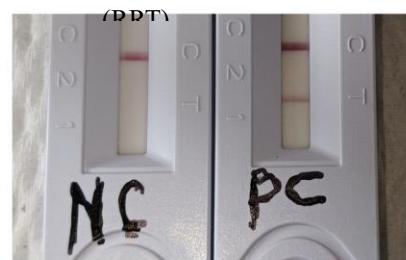
change in time-to-diagnosis; and (2) the effect on reported exposures subsequently receiving PEP, comparing the tiered diagnostic approach against the current standard of care. The implementation study will provide operational and cost-effectiveness data to inform the scale-up of the tiered diagnostic approach, measuring public health benefit through reducing delay to diagnoses (and thus enabling PEP)—in our experience, a major barrier to effective rabies prevention (Abubakar *et al.*, 2023; WHO Rabies Modelling Consortium, 2020).

Molecular Examination of RRT False-Negative Samples: Reverse Transcriptase-qPCR (RT-qPCR) testing will be conducted on the four false-negative samples included in this study (DFAT-positive, RRT-negative) to ascertain the concentration of rabies virus nucleoprotein antigen (viral load). This will further establish whether RRT efficacy was hampered by low viral load (a limitation of lateral flow tests) or had to do with the RRT not detecting specific, circulating super variant that might have alternative nucleoprotein epitopes (Kia *et al.*, 2018; Konzing *et al.*, 2023) that might affect RDT performance for any assays that develop avoidable influence of their conclusion on low sensitivity tests like RRT. These results will provide valuable data to factor into future RDT design and procurement.

End-User Feasibility and Acceptability Assessment: Perform a qualitative study (interviews and focus groups) examining view user feasibility, deemed reliability, and acceptability of the RRT for primary users. Although the RRT is relatively easy to use, the ability to successfully adopt the RRT into the One Health system depends on user-confidence, training, and existing work-flows (Blumberg, 2024; Kuye *et al.*, 2024). Although further research is needed to understand user acceptance, this will be fundamental for training and promoting the sustainability of the diagnostic tool.

VII. APPENDIX

A. Pictorial clips from the Rapid Rabies Test Kits

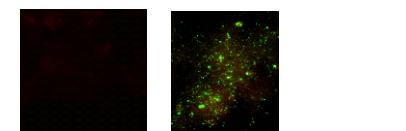


NC = Negative Control
 PC: Positive Control

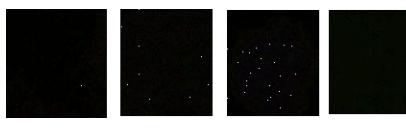


Some Selected Samples that Tested Positive to RRT.

B. Pictorial Clips of Direct Fluorescent Antibody Test (DFAT) Results



Negative Control Positive Control



Some Selected Slides of the DFAT Positive Samples

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