

WOAH Rabies Reference Laboratory Network's (RABLAB) overview of LFD tests for field application



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This document summarises the current knowledge of the WOAHP Rabies Reference Laboratory Network (RABLAB) on commercially available Point of Care (POC) tests. The mention of specific companies or products of manufacturers, and whether these have been patented, does not imply that these have been endorsed or recommended by WOAHP in preference to others of a similar nature that are not mentioned.

All commercial kits should be validated according to WOAHP's international standards. None of the POC tests included in this document are in the WOAHP Register nor are they as yet specifically described as a primary diagnostic test in the WOAHP *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*.

Introduction

Rabies is a contagious and deadly disease caused by lyssaviruses that target the central nervous system. As there are no gross pathognomonic lesions or specific, consistent clinical signs, rabies cannot be reliably distinguished from other neurological infections based on clinical presentation alone. Therefore, confirmatory laboratory diagnosis is essential. International standards for rabies diagnosis are described in 'Chapter 3.1.19. Rabies (infection with rabies virus and other lyssaviruses)' of the [Manual of Diagnostic Tests and Vaccines for Terrestrial Animals \(Terrestrial Manual\)](#) by the World Organisation for Animal Health (WOAH).

For animals that die following illness with signs consistent with rabies, timely diagnostic workup is essential. However, in resource-limited settings, logistical challenges often hinder sample submission to laboratories, or the laboratories may lack the capacity to perform internationally recognised diagnostic tests. As with other diseases, point-of-care (POC) tests offer an alternative by enabling rabies surveillance, facilitating rapid response, and increasing case detection.

Several POC tests – commercial rapid immunochromatographic tests, also known as lateral flow devices (LFDs) – for detecting rabies virus

(RABV) antigen are available on the market. These rapid test kits are simple to use, require no additional equipment or extensive training, and deliver results within minutes. Although these tests are not included in the WOAH certified Register of Diagnostic Kits and validated as fit for purpose, their use is not explicitly excluded. However, their application requires careful consideration.

Regarding test characteristics and specificities of RABV LFDs, the WOAH network of Reference Laboratories for Rabies (RABLAB) generally refers to the recent amendments in the [WOAH Terrestrial Manual](#).

This document summarises RABLAB's current knowledge of commercially available LFDs, including technical specifications, costs, and the advantages and limitations of each. The tests were selected either based on peer-reviewed publications that evaluated the tests or platforms, or based on independent assessments conducted in the authors' laboratories. It is important to emphasise that, while LFDs can serve as a useful adjunct to rabies diagnostics, they should not replace laboratory testing in national rabies control programmes.



Principle of the test

The test principle of LFDs is based on immuno-affinity partitioning, or immunochromatography, in which the analyte migrates laterally through a porous membrane towards a defined 'affinity' zone containing immobilised antibodies.

For rabies testing, a brain tissue suspension is applied to the sample port of the test cassette, where it is absorbed into the sample pad. The sample then flows through the conjugate pad, which contains anti-rabies antibodies (Abs) labelled with gold nanoparticles (**Figure 1**).

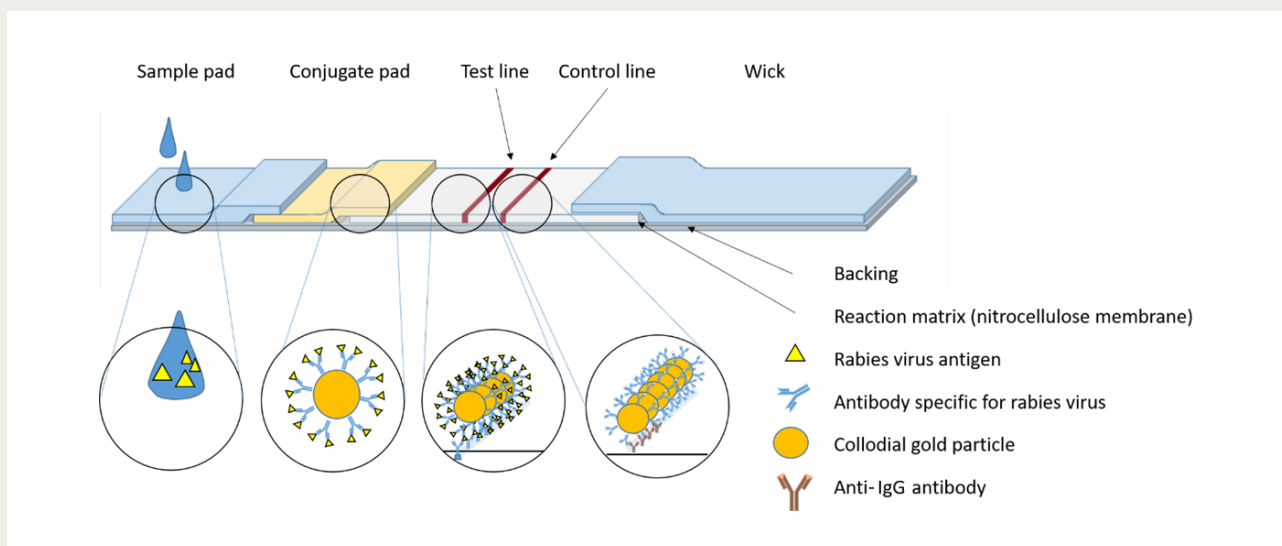


Figure 1. Pictorial representation of lateral flow device for the detection of rabies virus antigen

If a rabies virus antigen is present, nanoparticle-labelled antigen-antibody complexes migrate along the membrane strip and react with the test band ('T' line), which contains a second anti-rabies antibody. This interaction produces a visible coloured band (**Figure 2**). As the suspension continues to flow toward the control band, the anti-IgG coated at the 'C' line reacts with the nano

particle-labelled anti-rabies Abs, resulting in a control band. For the test to be valid, the reagents must flow correctly along the membrane strip, and the conjugate loaded with nanoparticles – either bound to antigen or unbound (as in a negative sample) – must react with the anti-IgG control line, confirming that the test has functioned correctly.



Figure 2. Photographs of a lateral flow device test showing a rabies-positive (left) and rabies-negative (right) result
Note: The internal control band only confirms that the detection antibody has migrated correctly along the strip. It does not verify the presence of the target antigen.

Factors influencing the performance of rabies LFDs

Unlike other WOAHA-recommended laboratory-based diagnostic methods, LFDs are ready-to-use kits. While this makes them convenient to use, it also limits the ability of laboratory experts to modify protocols.

As with any diagnostic test, it is essential to understand the capabilities and limitations of LFDs before use. Several factors can influence their performance:

- **Kit- or lot-specific factors:** These include the efficiency of detection labels, membrane quality, flow rate, signal amplification methods, as well as the clarity and consistency of the manufacturer's instructions.
- **Environmental and handling conditions:** These encompass storage temperature and humidity, compliance with instructions, operator skill in sample collection and the ease of interpreting results.

A common limitation of commercially available LFD kits is the absence of included positive and negative controls. Although each test strip contains an internal control line, this line only confirms that the detection Ab has flowed correctly along the strip; it does not function as a true positive control in the strictest sense.

The performance of antigen-based tests such as LFDs depends heavily on the characteristics of the anti-rabies detection Abs used and their binding affinity for the target antigen. This directly impacts the sensitivity and specificity of the test. Detection Abs should not cross-react with non-rabies antigens, as non-specific binding can lead to false-positive results. Because antibody affinity is variant-specific, the selection of appropriate detection Abs for an LFD is a critical determinant of which lyssavirus and RABV variants the test will detect. Given the global diversity of lyssaviruses, including RABV, the Abs used in these tests should be broadly reactive.

Similar to anti-rabies conjugates for the direct fluorescent antibody (DFA) test, anti-nucleoprotein (N) Abs offer higher sensitivity for detecting

RABV antigen compared to anti-glycoprotein (G) Abs. This is because the N protein:

- Is the most abundant structural protein in the RABV, making it easier to detect in infected tissues.
- Is highly conserved across different RABV strains, supporting broad-spectrum and reliable detection.
- Is produced early during viral replication, enabling earlier detection of infection than glycoproteins.

Modern commercial polyclonal anti-N Abs are generally more effective than monoclonal Abs (mAbs), as they can recognise all lyssaviruses. In contrast, anti-N mAbs may exhibit varying reactivity to certain lyssaviruses or even different variants of RABV. However, the use of a cocktail of two or more mAbs can overcome the limitations of individual mAb, thus substantially increasing the diagnostic sensitivity of LFDs.

Another important factor influencing LFD performance is the **limit of detection (LoD)**. Fortunately, the concentration of RABV antigen in the brains of rabid animals is typically very high, which generally poses no issue when testing under field conditions. However, in cases where animals are tested at the early onset of clinical signs, or when brain samples are suboptimal, the viral load may be low. In such situations, a lower LoD enhances diagnostic sensitivity by enabling the detection of even minimal amounts of RABV antigen.

The diagnostic sensitivity and specificity of an LFD should be estimated through accurate test validation, ideally conducted according to international standards. It is the manufacturer's responsibility to validate the LFD in line with these standards (see Chapter 1.1.6. of the [WOAH Terrestrial Manual](#)) to ensure consistent sensitivity and specificity for end users and to maintain high product quality. Furthermore, the instructions provided with the test kit should clearly reflect the procedures used during the validation process.

Should rabies LFDs be used for rabies diagnosis?

A common question posed by authorities is whether LFDs should be used for rabies diagnosis. In other words, do LFDs hold the same diagnostic value as other WOA-recommended tests?

Answering this requires careful consideration of the wide variety of LFDs currently available. Refer to **Table 1** for a full comparison.

Table 1. Comparison of three major test platforms for rabies virus detection

	Antigen detection		RNA detection
	Point of Care test	Laboratory	Laboratory
Test	Rapid test (Lateral flow device)	Direct Fluorescent Antibody Test (DFA)	Lab-based quantitative reverse transcription polymerase chain reaction (RT-qPCR)
Intended use	Screening test	Confirmatory test	Confirmatory test
Specimen type(s)	Brain tissue	Brain tissue	Brain tissue
Sensitivity	Variable	High	High
Specificity	Variable	High	High
Training	Minimal	Yes – specialised	Yes – generalised
Testing time	15 to 30 minutes plus brain biopsy collection	60 to 90 minutes plus brain biopsy and sample transportation time	60 to 90 minutes plus brain biopsy and sample transportation time
Costs/test (EURO)	7–10	10–15	20–30
Cost of equipment (EURO)	Not applicable	10,000 to 15,000	10,000 to 30,000+
Advantages	Rapid (early detection) Simple and easy to use (anyone can perform) No additional equipment needed Field application	High sensitivity and specificity	High sensitivity and specificity
Disadvantages	High variation in sensitivity Not a standardised method No positive/negative control run with the sample	Laboratory facilities needed Relatively high equipment cost	Laboratory facilities needed Relatively high equipment cost
Use	Screening test for surveillance	Stand-alone test for surveillance	Stand-alone test for surveillance
Comments	Available and new products need evaluation	Gold standard	Gold standard

To date, at least 17 different commercial rapid immunochromatographic tests (referred to in this text as LFDs) have been marketed for the detection of RABV antigen. In many evaluation studies, manufacturer instructions have been modified, which can significantly impact test performance. When assessing the sensitivity and specificity of a particular LFD assay, it is essential to consider any deviations from the manufacturer instructions and the potential impact on test performance. Some tests have even failed evaluations completely, while others remain unevaluated (Eggerbauer *et al.*, 2016, Klein *et al.*, 2020).

Although some reviews of test performance have provided sensitivity and specificity estimates for various LFDs, they cannot be recognised as being

fully validated in line with manufacturer's instructions. As such, they do not meet the expectations of end users relying on ready-to-use tests. For most LFDs, validation data are either unavailable or do not comply with the criteria outlined in Chapter 1.1.6. of the [WOAH Terrestrial Manual](#). Furthermore, there is often a lack of transparency regarding assay methods, particularly their composition and the reactivity of the detection Abs used.

Given the significant variability in the performance of commercial LFDs – and the serious public health risks posed by false-negative results due to low sensitivity – **WOAH RABLAB does not recommend the use of LFDs as a stand-alone diagnostic test for ruling out rabies infection.**

Are LFDs still useful?

Despite their limitations, WOAHRABLAB acknowledges the potential value of LFDs as screening tools to support rabies surveillance in endemic areas when access to WOAHR-recommended diagnostic tests is unavailable.

However, the appropriate use of LFDs should be determined by the competent authorities. The cost–benefit of incorporating these tests into rabies surveillance programmes remains under debate, and likely depends on specific programmatic objectives and the local rabies epidemiological context. If a country or Veterinary Authority has not accepted the LFD as an official test, then positive and negative LFD results should not be used for official surveillance statistics or formal rabies status designations. Conversely, if the country or Veterinary Authority has accepted the LFD as an official test, then positive results must be included in official surveillance statistics.

If LFDs are used as diagnostic tests for rabies surveillance, RABLAB recommends the following:

- **Use of only brain material for testing**
- **Use of only high-performance LFDs**, with a minimum of 90% sensitivity and 95% specificity. As of April 2025, two tests may meet these expectations (i.e. showing superior sensitivity) when the manufacturer's protocol is slightly modified by omitting pre-dilution of the sample (see **Table 2**).

- **Clearly define the purpose of the test**, such as surveillance or monitoring (screening) (**Table 3**).
- **Establishing a standardised protocol**, preferably following the manufacturer's instructions. If modifications are necessary, they must be rigorously followed.
- **Provide comprehensive training for staff**, including correct procedures for brain biopsy sample collection.
- **Demonstrate adequate LFD performance** for the RABV variants and animal species most relevant to the area where the test is used. This should include prior in-house evaluation by the national reference laboratory for rabies ([Annex 1](#)).
- **Never use a negative LFD result** to dissuade an exposed individual from receiving Post-Exposure Prophylaxis (PEP).
- **Confirm all negative test results** using WOAHR-recommended laboratory diagnostic techniques as outlined in Chapter 3.1.18. – Rabies (infection with rabies virus and other lyssaviruses) of the [WOAH Terrestrial Manual](#).
- **Report positive LFD results in line with World Health Organization and WOAHR standards**, which may require confirmation using an officially recognised test.
- **Ensuring proper biosecurity, PPE use and sample collection training** for all test users ([Annex 2](#)).

Given the large number of LFDs for rabies on the market, careful selection is critical. To date, only two LFD tests have been extensively studied

and approach the recommended thresholds for diagnostic sensitivity and specificity (**Table 2**).

Table 2. Comparison of two LFD tests for rapid rabies virus antigen detection with supporting validation data indicating potential applicability

Test	Antigen Rabies Ag detection rapid test		ADTEC Rabies Ag Test Kits	
Manufacturer	Bionote, Animal Genetics, Inc., Gyeonggi-Do, Korea (Rep. of)		ADTEC, Oita, Japan	
Catalogue no.	RG1801DD		No information	
Website	https://www.bionote.co.kr/index_en.html		No information	
Specimen type	Brain tissue		Brain tissue	
Species listed on package insert [†]	Canine, bovine, raccoon dog		Canine	
Format	Lateral flow device		Lateral flow device	
No. of mAbs used/affinity	1 / RABV nucleoprotein (N) specific		2 / RABV nucleoprotein (N) specific	
Level of assessment	<ul style="list-style-type: none"> - Peer-reviewed published journal article - Independent assessment at Reference Laboratories 		<ul style="list-style-type: none"> - Peer-reviewed published journal article - Independent assessment at Reference Laboratories 	
Procedure	1/10 pre-dilution*	Without pre-dilution**	1/10 pre-dilution step	Without pre-dilution*
Overall sensitivity	60.8–100%	94–100%	74–95.5%	94–96.3%
Specificity	93–100%	93.3–100%	88.9–100%	100%
Reported sensitivity	Kang et al. (2007): 91.7% Markotter et al. (2009): 100% Yang et al. (2012): 95% Servat et al. (2012): 88.3% Reta et al. (2013): 96.5% (95% CI: 90.0–99.3%) Voehl and Saturday (2014): 96.9% Sharma et al. (2015): 91.66% Ahmad and Singh (2016): 85.7% Eggerbauer et al. (2016): 60.8% Dohmen et al. (2018): 97.96% Servat et al. (2019): 99.5% Tenzin et al. (2020): 92% Klein et al. (2020): 62% Aparna et al. (2022): 96.6%	Léchenne et al. (2016): 95.3% Chandra et al. (2017): 100% Certoma et al. (2018): 100% Yale et al. (2019): 96% Mauti et al. (2020): 98.2% Alvarado-Fernández et al. (2023): 94%	Nishizono et al. (2008): 93.2–95.5% Ahmed et al. (2012): 74–95% Kimitsuki et al. (2020): 88%	Kimitsuki et al. (2020): 94% Mananggit et al. (2021): 94.3% Cruz et al. (2023): 96.3% Moh'd et al. (2024): 95% Todoroki et al. (2025): 100%

*Current manufacturer's instructions, **Modification of manufacturer's instructions [†]Cross-reactivity and false positives have been reported in rabbits with BioNote. Users should exercise caution when interpreting results in species not listed on the package insert. In-house evaluation is strongly recommended (see Annex 1).

Table 3. RABLAB recommended application of LFDs as a stand-alone diagnostic test in rabies endemic, low-income regions

Purpose	LFD result	Immediate actions	Laboratory confirmation	Laboratory result	Final interpretation	Deferred actions
Animal testing in the context of a biting incident	Positive	Inform authorities Initiate post-exposure prophylaxis (PEP)	Not necessarily required*	Not applicable	Positive case	None
	Negative	Inform authorities Conduct WHO Risk assessment and initiate PEP if indicated	Immediately required	Positive	Positive case	Inform authorities PEP needs to be completed
				Negative	Negative case	None PEP can be discontinued
Screening in the context of passive surveillance (including wildlife)	Positive	Inform authorities	Not necessarily required*	Not applicable	Positive case	Inform authorities
	Negative	None	Recommended	Positive	Positive case	Inform authorities
				Negative	Negative case	None

*If the country or Veterinary Authority has not recognised the LFD as an official test, then neither positive nor negative LFD results should be used for official surveillance statistics or formal rabies status designations. Conversely, if the country or Veterinary Authority has accepted the LFD as an official test, then positive results must be included in official surveillance statistics.



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Annex 1. Minimum requirements for in-house test evaluation

When using an LFD as a screening tool, it is imperative to assess whether the test can reliably detect the rabies virus lineages prevalent in the country. Although this process does not constitute full validation, it provides valuable information for competent authorities to determine whether the test is fit for its intended purpose. The number of samples required for in-house verification depends on the desired levels of sensitivity, specificity and statistical confidence.

The following example serves as a general guideline for in-house verification of LFD performance:

To assess a diagnostic test with a sensitivity (S_n) of 97% and a specificity (S_p) of 99%, both positive and negative samples must be included. A S_n (True Positive Rate) of 97% means that 97% of actual positive samples are correctly recognised as positive. On the other hand, a S_p (True Negative Rate) of 99% means that 99% of true negative samples are correctly identified as negative.

To confirm sensitivity of 97% and specificity of 99%, with a margin of error of $\pm 5\%$ at a 95% confidence level, at least **45 positive** and **15 negative** samples should be tested, with 100% agreement between the LFD and a gold standard test. In practice, the number of negative samples can be increased (up to four times the number of positives), as they are typically easier to obtain.

Additional practical considerations include:

- **Positive samples:** These should ideally represent a homogeneous distribution from all parts of the country and include known or putative reservoir species.
- **Prevalence:** The proportion of positive and negative samples should reflect the expected prevalence of the disease in the target population.
- **Statistical confidence:** While increasing the number of samples improves statistical confidence, it does not alter the fundamental interpretation of sensitivity and specificity (see **Figure A1**).

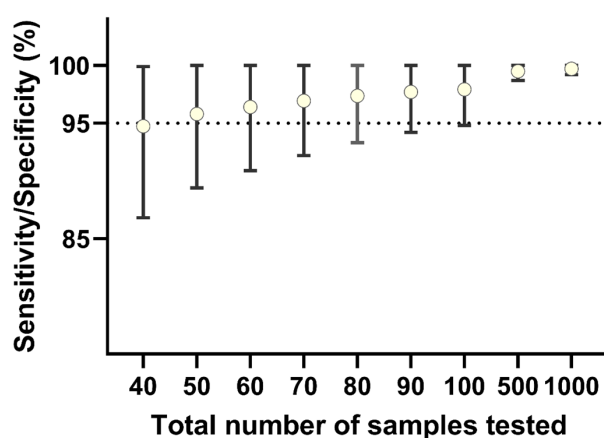


Figure A1. Influence of the number of samples tested on sensitivity and specificity if there is a single false positive/negative test result

Bars represent the 95% confidence intervals.

Since positive and negative samples are not typically tested together in a single session, attention must be given to factors that may affect kit quality. In this context, RABLAB recommends that the national Reference Laboratory validates each new batch of LFDs using a reference panel (including

both negative and positive samples) before releasing the kits for field use.

For further information on the validation of diagnostic tests, please refer to Chapter 1.1.6. (Validation of Diagnostic Assays for Infectious Diseases of Terrestrial Animals) of the [WOAH Terrestrial Manual](#).

Annex 2. General safety considerations

Biosafety precautions for the use of LFDs must adequately address all reasonable concerns related to rabies virus exposure. Personnel involved in rabies testing should be fully informed of the potential risks associated with brain sample collection and the test procedure itself, and should know how to mitigate these risks before initiating any rabies testing. All specimens must be treated as potentially infectious

A risk assessment should be conducted prior to testing, taking into account:

- The vaccination status of laboratory personnel who carry out the testing (i.e. pre-exposure rabies immunisation and antibody titre monitoring).
- The specific protocols involved (e.g. brain sample collection, test protocols, waste disposal and decontamination).
- The personal protective equipment (PPE) required.

For practical purposes, a distinction should be made between LFD use under field conditions and use within a biosafety cabinet. Under field conditions, the recommended minimum PPE includes a lab coat, gloves, FFP2 masks and safety glasses or face shields.

Whenever possible, disposable materials and devices should be used for LFD testing. However, if reusable equipment is employed, it must be thoroughly decontaminated with an approved disinfectant after use. All disposable materials should be incinerated or autoclaved before disposal.

Competent authorities may implement additional biosafety measures if deemed necessary to reduce the risk of rabies virus exposure.

LFDs are designed as rapid field tests intended to be used easily and safely, provided that the aforementioned biosafety recommendations are strictly followed.



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