

## Original Research Article

## Evaluation of Lateral Flow Assay on Skin Specimen for Scaling up Canine Rabies Surveillance

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**Abstract:** Surveillance of animal rabies is severely constrained in resource poor developing countries. The traditional reference method of brain testing has several limitations. Hence there is need for alternative method which is rapid, simple, sensitive and specific on a less invasive sample to scale up rabies surveillance. The present study evaluated the efficacy of Lateral Flow Assay (LFA) on skin specimen for post mortem screening of rabies in dogs. Skin samples and brain tissue of 56 dogs including two wild dogs which came for routine rabies surveillance were collected at necropsy. LFA on skin, LFA on brain and Fluorescent Antibody Test (FAT) on brain were conducted. The diagnostic efficacy of LFA on skin was compared against FAT on brain, reference method for rabies. Correlation between LFA on skin and LFA on brain was also evaluated. The study found that LFA on skin has equal efficiency as that of LFA on brain for post mortem detection of rabies in dogs. It revealed a sensitivity of 96.6% and specificity of 100% against the traditional reference method of brain testing by FAT. Based on the results, LFA on skin looks promising as a practical field tool to improve reporting and strengthen surveillance. The findings warrant further evaluation on a large sample set under different field conditions.

**Keywords:** Skin Specimen, Direct Fluorescent Antibody Test, Lateral Flow Assay.

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## INTRODUCTION

Surveillance of animal rabies is severely constrained in developing countries. Lack of availability of field level diagnostic tools, biosecurity issues in collection of samples, poor laboratory facilities in the peripheral levels and various other factors critically cripples laboratory based surveillance. There is an urgent need to scale up canine rabies surveillance worldwide to meet the target of zero rabies by 2030. Lateral Flow Assay (LFA) is a simple test that aids rapid diagnosis of rabies in the field without the need for expensive laboratory instruments and human expertise. There are many studies evaluating efficacy of different commercially available Lateral Flow Devices (LFDs) with variable sensitivity [1, 2]. Though implementation of these devices has improved surveillance in resource-limited settings, a real breakthrough has not been materialized so far as the

traditional gold standard method of brain testing has several limitations. Availability of reliable samples other than brain tissue would circumvent the need for invasive procedures and safety issues.

Nuchal skin biopsy are proved to be intra-vitam diagnostic for rabies in humans and animals. There are a few studies evaluating the potential of skin specimen in post mortem diagnosis in animals. In this preliminary study, we evaluate diagnostic efficiency of a commercially available lateral flow device using skin specimen collected at necropsy. To our belief, this is the first study evaluating diagnostic accuracy of LFA on skin samples.

## MATERIALS AND METHODS

Rabies suspected dogs submitted to State Institute for Animal Diseases, Kerala, India for

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necropsy is included in the study. The total number of dogs received during this study period was 56. Brain and nuchal skin samples were collected in each case. LFA on brain and skin samples were conducted side by side. Anigen Rapid Test (commercially by Bionote) which is a validated LFD by many studies [1, 2] was used in this trial. Brain samples were evaluated further by Fluorescent Antibody Test (FAT).

Technical performance of LFA on skin was compared with LFA on brain and FAT on brain.

**Brain sample collection**

The skull was opened to expose the brain and the brain was collected fresh using all bio-security precautions. Brain sample were collected which comprised of a composite mixture of the brain stem, hippocampus and cerebellum.

**Skin sample collection**

The skin samples were collected from nape of the neck region<sup>4</sup>. Section of the skin with a 2cm X 2 cm and was taken ensuring that section comprised a minimum of 10 hair follicles. The skin biopsy was taken at a sufficient depth in the subcutaneous plane to include the nerves at the base of the hair follicle. The skin was finely triturated using motor and pestle and sampled.

**Lateral Flow Assay**

The test was done according to instructions described in the manual. Collected the samples from skin triturates, and brain homogenates using the sterile swab supplied with the kit taking care to see that approximately 1-2 gm of the sample is taken. Inserted the swabs into the specimen tube containing 1ml of assay diluents in 1: 10 dilution. Mixed the swab samples with assay diluents and allowed to stand for a few minutes for the coarse particles to settle.. Removed

the test device from the foil pouch, and placed it on a flat and dry surface. Using the disposable dropper provided, added four (4) drops of supernatant into the sample hole using the disposable dropper. Interpreted the test results at 5 ~ 10 minutes as described in the manual.

**Direct Fluorescent Antibody Test (DFAT)**

DFAT was carried out according to Meslin *et al.*, [3]. Impression smear preparations of the brain samples were placed in a Coplin jar containing chilled acetone and fixed at 4°C for one hour. The slides were air-dried and incubated with anti-rabies nucleocapsid conjugate (Bio-Rad, France) against rabies for 35 min at 37°C in a humid chamber and further washed with phosphate buffered saline (PBS) in two successive washes for 5-10 min, air-dried and mounted with buffered glycerol and then visualized under an immune fluorescent microscope (Olympus) at ×400 magnification. Bright/dull/dim apple-green round to oval intracellular accumulations were observed and recorded according to Tepsunmethanon *et al.*, 1997 [4].

**RESULTS**

Table 1 describes the performance of LFA on skin sample, brain sample in comparison with FAT on brain sample. Of the 56 canine cases tested which included two wild dog samples also, 30 brain samples were positive on FAT (Fig 1) and 29 each on LFA in skin (Fig 2) and brain sample (Fig 3). Taking FAT as gold standard, sensitivity LFA on skin was 96.6%. All samples negative on FAT were negative on LFA on skin also demonstrating a specificity of 100%. LFA results on skin and on brain were in concordance though there was difference in the intensity of band in majority of cases. Intensity of the band and development of band after the stipulated time are considered not relevant.

**Table 1: Diagnostic performance of LFA on Skin**

Tested	LFA on Skin		LFA on brain		FAT on brain (Gold standard)	
	Positive	Negative	Positive	Negative	Positive	Negative
56	29	27	29	27	30	26

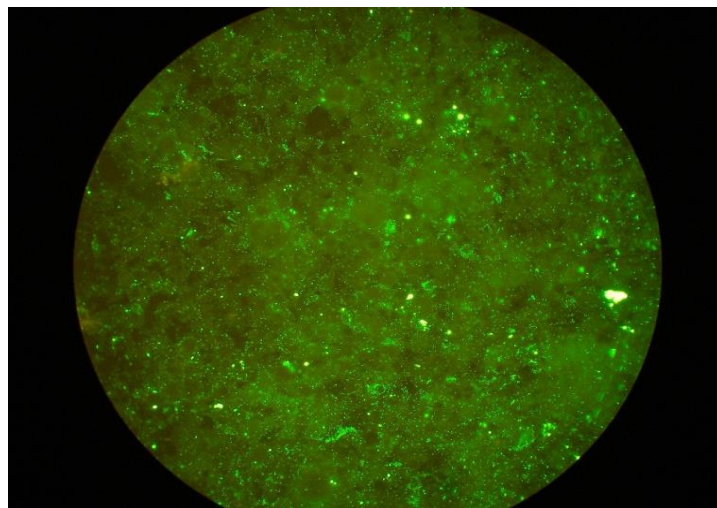
**Table 2: Cross comparison of LFA on Skin with FAT on Brain**

		FAT on Brain		
		Positive	Negative	Total
LFA on Skin	Positive	29	0	29
	Negative	1	26	27
	Total	30	26	56 (n)

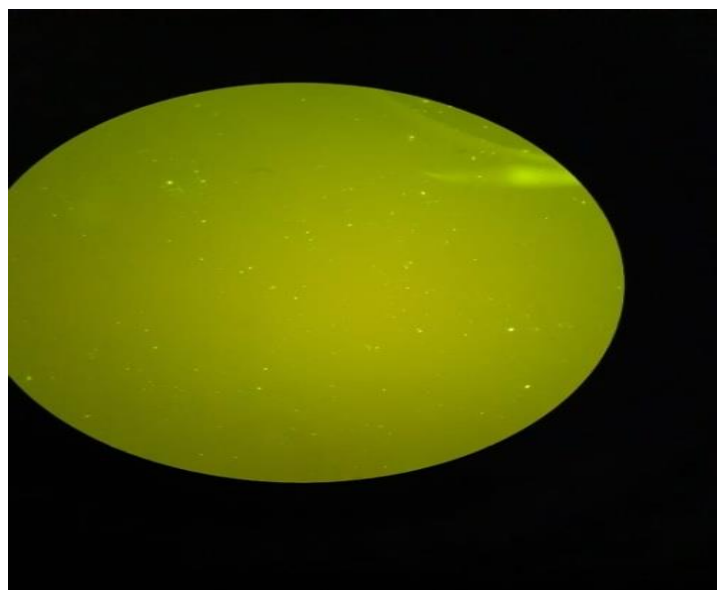


**Fig 1: Brain and skin specimens on LFD**

Figure 1 Brain and Skin specimens on DFAT as visualized under Olympus BX51 Fluorescent microscope.



**Fig 2: Brain specimen on FAT (+++) fluorescence**



**Fig 3: Skin specimen on FAT(+) fluorescence**

## DISCUSSION

In a period of concerted efforts towards the control, elimination and eradication of rabies the role of effective, decentralized laboratory testing becomes increasingly important. Identification of rabies viral RNA in any tissue is diagnostic of rabies infection. Point of care or animal side devices which are simple, reliable and easy to operate on easily accessible non invasive sample would be an ideal approach for improving surveillance [5]. This paper presents the result of the first study conducted to investigate the suitability of LFA, a widely accepted practical field tool, on skin specimen, a superficial handy sample, to diagnose canine rabies. The study revealed a sensitivity of 96.6% and specificity of 100% in comparison with traditional gold standard method, FAT on brain. 100% concordance found between LFA on skin and LFA on brain proves that LFA on skin has equal potential to detect rabies as that of brain raising hope for promoting it as an animal side field level tool to scale up canine rabies surveillance.

It is pragmatic to believe that virus load in skin would be lower in comparison to brain and hence sensitivity of the test is likely to decline at low virus load as it may happen in ante-mortem samples and animals euthanized before full run of clinical course. We recommend further studies to evaluate the test in a large sample set with different time of collection and sites of skin sampling. There is evidence that skin sample of muzzle of dogs has more viral load due to high innervations [6]. Other limitations include varying intensity of test band in comparison to control line which could be interpreted otherwise. However this study demonstrated that skin has sufficient antigenic load to bind with antibodies in the test strip for a visible line to appear at the test line position if applied as a post mortem tool.

## CONCLUSION

The yielded promising results of the efficacy of Lateral Flow Assay on skin sample as a post mortem rabies screening tool with potential to act as a game-changer in canine rabies surveillance in resource poor countries.

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