Title: EVALUATION OF LATERAL FLOW ASSAY AS A PERIPHERAL LEVEL DIAGNOSTIC TOOL FOR RABIES IN ANIMALS

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Keywords

Abstract

Rabies continues to be a significant public health problem in many Asian, South American and African countries. India alone accounts for about 20000 human deaths annually as per the latest reports. Though human rabies is nearly 100& fatal, it is instituted particularly in people exposed to bites from proven rabid animals.

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Evaluation of Lateral flow assay as a Peripheral Level Diagnostic tool for Rabies in Animals

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Introduction

Rabies continues to be a significant public health problem in many Asian, South American and African countries. India alone accounts for about 20,000 human deaths annually as per the latest reports. Though human rabies is nearly 100% fatal, it is effectively preventable if prophylactic measures are instituted particularly in people exposed to bites from proven rabid animals. Laboratory confirmation of rabid status of the biting animals is essential for proper medical decisions regarding the treatment of individuals potentially exposed to rabies. A number of laboratory tests have been standardized internationally for diagnosis of rabies such as Negri body detection, Fluorescent Antibody Test, Mice inoculation, Enzyme immuno assays and Molecular methods each having its own merits and demerits. However all these tests require lot of expertise and expensive equipments and are generally done in reference laboratories. Hence in this study, a commercially available rabies antigen detection kit based on lateral flow technique is evaluated for its adaptability in peripheral centers under field conditions without compromising on the specificity and sensitivity. The Direct Fluorescent Antibody Test, the accepted gold standard technique for routine rabies diagnosis (Chabra et.al., 2007; Madusudana et al., 2003), was employed as reference test in this evaluation.

Materials and Methods

A total of 88 brain samples of different animals viz. canine(63), feline(13), bovine(4), caprine(7), squirrel(1) and one dog saliva sample collected from animals suspected to have died of rabies brought to this laboratory for confirmation during the period April'08 to December'08 were included in this study.

Direct Fluorescent Antibody Test (DFAT) : The test was conducted as per the technique described earlier (OIE, 2000) with a few modifications. A set of impression smears were prepared from brain stem, cerebellum and hippocampus. After air drying, the smears were fixed in chilled acetone at -20°C overnight before being stained with rabies antinucleocapsid fluorescent conjugate (BIORAD, Cat. No.72112). The slides were incubated for 45 minutes at 37°C in a humid chamber and were then washed with PBS of pH 7.4 in three successive 5min wash periods. The slides were then air dried, mounted in 0.05M Tris buffered saline of pH 9.0 with 20% glycerol and examined at 400X on a fluorescent microscope (Olympus CX 41).

Lateral flow assay (Rapid Antigen Detection Kit): The test was conducted according to manufacturer's (M/s Anigen Animal Genetics, Inc., Korea, Cat No.RG 18-01) instructions. A 10% brain homogenate (stem, cerebellum and hippocampus) was prepared in sterile PBS and inoculated in the sample vial supplied along with the kit. Mixed and kept for 10sec, 5 drops of the supernatant was added to the sample window and results were taken after 10min. The saliva was directly inoculated in to the sample vials.

Results and Discussion

Of the 88 brain samples tested for rabies, 42(47.7%) were found positive by direct fluorescent antibody test (Fig.1) and 40 samples were positive by the kit method (Fig 2). The results of these tests are depicted in the Table. The diagnostic sensitivity and specificity of the kit was found to be 93 % and 98% respectively. It can be seen that the overall concordance between these two tests was 96%.

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Fig. 1

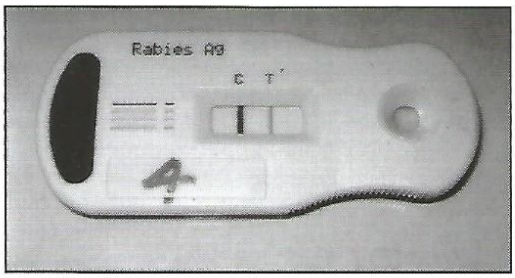

Fig. 2

However the saliva sample was negative on rapid antigen detection method, where as brain of the same dog when tested after death was strongly positive on Lateral flow assay (2+) and by FAT (3+ degree of fluorescence). The low antigen concentration in the saliva could be reason for the false negative result. Moreover it is well documented that the presence and concentration of virus in the saliva vary in rabid dogs (Frederick et al., 2000). The brain samples that were found negative on the kit but FAT positive had very scanty distribution of viral antigen (1+ degree of fluorescence). These findings suggest that the antigen concentration in the sample is important for the test to detect the infection and so it could be more applicable on brain samples than on samples with low antigen mass like saliva, CSF etc.

Table

Results of Lateral flow assay (LFA) on brain samples compared with fluorescent antibody test (FAT)

		FAT		
		Positive	Negative	Total
LFA	Positive	39	1	40
	Negative	3	45	48
	Total(n)	42	46	88
Sensitivity – 92.8%				
Specificity – 97.8%				
Concordance – 95.5%				

Lateral flow assay, an immuno chromatographic technique employing capture antibody and detector antibody is an established procedure with availability of commercial kits for the rapid detection of many viral, bacterial and toxic components. However this technique is yet to be used widely in the field of rabies. In this study, the commercially available kit has shown 96% correlation with the gold standard FAT with brain as sample in rabid animals. Good specificity and sensitivity were also observed. To conclude, the kit evaluated here is a rapid, simple, specific and effective tool for peripheral level laboratory diagnosis of rabies in animals using post mortem brain specimens. It is imperative, however, that FAT or virus isolation procedures be used to confirm all negative test results because of the observed discrepancy in the sensitivity.

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