



Sensitivity comparison of four diagnostic techniques to detect street rabies virus

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Prompt and accurate diagnosis is of utmost importance for the surveillance and control of rabies in man and animals. The specific detection of viral antigen by immunofluorescence (Dean and Abelseth 1973) was an accurate method for diagnosis of rabies (Tepsumethanon *et al.* 1997). Another rapid test, counter immunoelectrophoresis was projected by Diaz and Varela- Diaz (1976). Whitby *et al.* (1997) concluded that reverse transcriptase –polymerase chain reaction is useful and easy to perform, rapid and highly sensitive tool for the detection of rabies. In developing countries where rabies is endemic, not every laboratory can afford to be equipped with costly sophisticated equipments. Therefore, alternate methods which are rapid, reliable, economic and easy without depending upon sophistication have to be developed. With this view a study was undertaken to evaluate the sensitivity of direct immunofluorescence test (FAT), counter-immuno electrophoresis test (CIEP) histopathology and impression smears stained with Seller's staining in the diagnosis of rabies

Street rabies virus (SRV) was isolated (Meslin *et al.* 1996) from a rabid 3-months-old male dog with the history of aggressive behaviour. The SRV isolate was subjected to 2 passages in mice brain to accomplish multiplication of SRV. LD₅₀ of SRV isolate was calculated as per Reed and Muench (1938). The inoculum was prepared as per Sehgal *et al.* (1987). The infected mouse brains were pooled, weighed and homogenized in a sterile pestle and mortars to make a fine paste. Chilled phosphate buffer saline sterile solution was used as diluent to make a 10% suspension and mixed thoroughly and centrifuged at 1000 rpm for 5 min. Penicillin (100 IU/ml) and streptomycin (50 mg/ml) were added to the supernatant and kept at 4°C for 30 min.

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The inocula in different groups were treated for 30 and 60 min at different ranges of temperature and exposed to sunlight for 30 and 60 min in different weather conditions. The exposure of inocula to temperature was achieved in the incubator. The effect of sunlight was observed on SRV in different months of the year corresponding to different weather conditions. The experimental studies of cold weather (10–20°C) was performed during December that of moderate weather (20–32°C) during April and of hot weather (32–37°C) during May (Table 1). Temperature of weather was recorded with the help of maximum–minimum temperature thermometer.

Albino mice (180) were divided into 5 sub-groups, viz. sub group 1 – 5 (Table 1) to analyze the effect of temperature treatment and sunlight exposure on SRV. 10% suspension of SRV (Sehgal *et al.* 1987) was inoculated with 3090 MICLD₅₀ of SRV (0.03 ml /mice) by intra-cerebral route. Six mice each were inoculated with SRV treated with different temperature treatments and exposed to sunlight in cold weather (10–20°C), moderate weather (20–32 °C), hot weather (32–37 °C), hot and dry weather (37–40 °C) and hot and humid weather (37–40 °C) for the durations of 30 min and 60 min. In addition, 6 mice each were inoculated with SRV without any exposure to temperature and sunlight that served as positive control. Finally, 6 mice were inoculated with 10% normal mice brain suspension in PBS without any exposure to temperature and sunlight that served as negative control.

The mortality pattern after infection of albino mice was analysed at 3 stages of mortality, viz. start of mortality, 50% mortality and 100% mortality. Various diagnostic techniques, viz. direct FAT (Meslin *et al.* 1996) on impression smears of various nervous tissues, CIEP (Asha Rani *et al.* 2002), histopathology, Impression smears stained with Seller's staining (Sehgal *et al.* 1987) were compared for the sensitivity of detection of rabies. The % sensitivity of various tests with FAT was calculated by applying method of Perrin and Sureau (1987).

Sample tested	CIEP positive, FAT positive, FAT negative	CIEP negative, FAT positive, FAT negative,
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$$\text{Sensitivity} = \frac{\text{True positive}}{\text{True positive} + \text{False negative}} \times 100 = \%$$

Statistical analysis of data was done by using Kolmogorov-Smirnov one sample non parametric test. All the groups were compared with the positive control at 1% level of significance as reported by Sidney and Castellan (2002).

SRV isolate was confirmed by various techniques (Table 2), viz. Seller's staining in impression smear of hippocampus showing presence of Negri bodies, FAT revealed apple green fluorescence in impression smear of hippocampus, cerebrum and cerebellum, histopathological examination of hippocampus revealed Negri bodies and mice inoculation test in which mice inoculated with SRV showed ruffled hair coat, tremors and paralysis followed by death within 9–12 days. Isolation of SRV from rabid dog was similarly reported by Jayakumar *et al.* (1990) and Archana *et al.* (2003). The MICLD₅₀ was 10^{3.49}/0.03 ml of inoculum, which was in accordance with the finding of Singh and Grewal (1998).

In infected brain tissues of albino mice in different subgroups % sensitivity of CIEP with FAT for detection of rabies antigen was 82.66% (Table 3). Similarly, comparison

of % sensitivity of impression smears stained with sellers staining with FAT in different sub-groups for detection of rabies antigen was found to be 39.33% (Table 3). However, comparison of % sensitivity of histopathology with FAT in different subgroups for detection of rabies antigen was found to be 52% (Table 3).

Among these techniques, on the basis of comparison of % sensitivity of FAT in different sub-groups with various diagnostic techniques. CIEP was found to be the most sensitive technique for the detection of rabies antigen from the various brain tissues of infected albino mice followed by histopathology. Shankar (2009) reported that the sensitivity of histological techniques is much less than that of immunological methods, especially if there was some autolysis of the specimen. Whereas, impression smear stained with sellers staining technique was found to be the least sensitive. The findings in this study are in accordance with other findings (Jayakumar and Ramadassa 1991, Dutta *et al.* 1992, Tepsumethanon *et al.* 1997, Qureshi *et al.* 2003). Similarly, Praveena *et al.* (2003) compared the efficacy of fluorescent antibody technique (FAT), cytological examination and histopathological examination in diagnosis of rabies and concluded that FAT was highly sensitive and cytological examination remained the least sensitive followed by the histopathological examination. Whereas, Archana *et*

Table 1. Experimental design

Sub-groups	Treatments				Control		Dose of inoculum
	Temperature		Sunlight		Positive	Negative	
	Time duration (min)		Time duration (min)		No. of mice	No. of mice	
1 Cold weather (10–20°C)	30	60	30	60	6	6	3090 MICLD ₅₀
2 Moderate weather (20–32°C)	30	60	30	60	6	6	3090 MICLD ₅₀
3 Moderate hot weather (32–37°C)	30	60	30	60	6	6	3090 MICLD ₅₀
4 Hot and dry weather (37–40°C)	30	60	30	60	6	6	3090 MICLD ₅₀
5 Hot and humid weather (37–40 °C)	30	60	30	60	6	6	3090 MICLD ₅₀

SRV: 10% Street rabies virus suspension in phosphate buffer saline.

Table 2. Diagnosis of rabies in brain tissues of albino mice (each 6 per treatment) infected with street rabies virus (SRV)

Treatments	No. of albino mice brain tissues found positive for rabies																			
	Sub group 1				Sub group 2				Sub group 3				Sub group 4				Sub group 5			
	FAT	CIEP	IS	H	FAT	CIEP	IS	H	FAT	CIEP	IS	H	FAT	CIEP	IS	H	FAT	CIEP	IS	H
T30	6	6	3	3	6	6	3	4	6	6	3	4	6	6	3	4	6	6	3	3
T60	6	6	3	4	6	5	3	3	6	6	3	3	6	5	3	3	6	5	2	3
TS30	6	6	2	3	6	6	2	3	6	5	2	4	6	5	2	3	6	5	2	4
TS60	6	5	2	3	6	5	2	4	0	0	0	0	0	0	0	0	0	0	0	0
PC	6	6	3	4	6	6	4	4	6	6	2	4	6	6	4	4	6	6	3	4
NC	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

T30, Temperature 30 min; T60, temperature 60 min; TS30, temperature and sunlight 30 min; TS60, temperature and sunlight 60 min; PC, positive control; NC, negative control; FAT, fluorescent antibody test; CIEP, count-immuno-electrophoresis; IS, impression smear; H, histopathology; mnDm=36**.

**Control versus temperature and sunlight 60 min is significant at 1%.

Table 3. Comparison of sensitivity of CIEP, impression smear and histopathology with FAT for detection of rabies antigen in brain tissues of albino mice infected with street rabies virus (SRV)

Sample tested	CIEP positive		CIEP negative		Impression smear positive		Impression smear negative		Histopathology positive		Histopathology negative	
	FAT positive	FAT negative	FAT positive	FAT negative	FAT positive	FAT negative	FAT positive	FAT negative	FAT positive	FAT negative	FAT positive	FAT negative
150	124	0	26	18	59	0	91	18	78	0	72	18
Sensitivity %*	82.66				39.33				52			

$$\text{*Sensitivity \%} = \frac{\text{True positive}}{\text{True positive} + \text{False negative}} \times 100$$

al. (2003) compared different diagnostic techniques such as Seller's staining, direct FAT, ELISA, mice inoculation test and histopathological detection of Negri-bodies, and found ELISA the most sensitive technique followed by direct FAT, Seller's staining, MIT and histopathological detection of Negri-bodies. However, Singh and Grewal (1998) had compared different diagnostic techniques to detect rabies virus antigen/antibody and reported that detection of neutralizing antibodies by using modified counter immunoelectrophoresis was the most sensitive technique followed by direct immunofluorescence; Seller's staining of Negri body, and mice inoculation test. The least sensitive of all the diagnostic technique was the histopathological detection by Negri-body.

SUMMARY

Experimentally infected, suckling albino mice (180) were inoculated by intra-cerebral route with 3090 MICLD₅₀ of street rabies virus, isolated from rabid dog, treated for 30 and 60 min at different ranges of temperature and exposed to sunlight for 30 and 60 min in different weather conditions, to evaluate the sensitivity of direct immunofluorescence test, counter-immuno electrophoresis test, histopathology and impression smears stained with seller's stain to detect street rabies virus. Among these techniques, on the basis of comparison of per cent sensitivity of FAT in different sub-groups with various diagnostic techniques. CIEP was found to be the most sensitive technique for the detection of rabies antigen from the various brain tissues of infected albino mice followed by histopathology. Whereas, impression smear stained with sellers staining technique was found to be the least sensitive.

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