Website : www.asthafoundation.in
Progressive Research : An International Journal
Vol 18 (1) : 51-53 (January-2023), Print ISSN : 0973-6417, Online ISSN : 2454-6003
Society for Scientific Development in Agriculture and Technology, Meerut (U.P.) India

Determination of NSP Antibodies to FMD Virus in Serum Impregnated in Filter Paper

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Abstract

Serum is a specimen of choice for various diagnostic tests, especially for serodiagnosis. However, it is of critical importance to collect and transport serum to the laboratories without being exposed to variable conditions such as temperature fluctuations and contamination. A study has been conducted to determine the applicability of filter papers for collection and transport of serum samples. A total of 100 known positive and 100 known negative DIVA (Differentiation of Infected from Vaccinated Animal) tested serum samples, for the presence of anti-non- structural protein antibodies against FMDV, were impregnated in pre-cut filter paper. Both the raw and filter paper-blotted serum samples were examined for presence of non-structural protein antibodies to FMD virus. Also, three different buffers were used for elution of serum antibodies and the sensitivity and specificity of r3AB(3) indirect ELISA was evaluated for detection of NSP antibodies. Results revealed that the diluents buffer supplied by ICAR PD-FMD, Mukteswar (UK) along with the r3AB(3) ELISA kit (sensitivity 97%; specificity 100%) was found to be more suitable for elution of serum antibodies from blotted filter paper than PBST (sensitivity 94%; specificity 88%) and PBS (sensitivity 92%; specificity 83%). The positive and negative predictive values were found to be 100% and 97.09 % for blotted serum samples eluted using diluents buffer, 88.68% and 93.62% for PBST and 84.40% and 92% for PBS, respectively.

Key word : *FMD, antibody detection, filter paper, serum, NSP.*

Introduction

Foot and mouth disease (FMD) is an extremely contagious, acute viral disease of the cloven hoofed animals and is known to be endemic in India and the SAARC sub-region. To identify the FMD endemic pockets in a particular area, the sero-prevalence study of Foot-and-Mouth Disease (FMD) virus is of paramount importance. Differentiation of Infected from Vaccinated Animal (DIVA) principle, as stated by (1) exploits differences in the antibody response generated in vaccinated animals compared to animals having natural FMD virus infection. FMD vaccines contain only structural proteins (SP) of the virus and the NSP are deliberately removed. In contrast, during natural infection the antibody generated contains antibodies against both structural and non-structural proteins of the virus and hence presence of the antibodies against NSPs in serum samples refer to natural infection and absence of the same is an indication of vaccinated animal. Diagnostic accuracy plays a central role in the evaluation of diagnostic tests. Test accuracy may be expressed as sensitivity and specificity or as positive and negative predictive value (Parikh *et al*, 2008). Collection and transport of serum samples for accurate sero-diagnosis of FMD can be highlighted as a crucial step, as it is affected by many factors including poor packaging, contamination and temperature variations (2). In order to tackle this problem, it is important to select an efficient serum collection method. In this study, the specificity as well as sensitivity of use of filter papers in serum sampling method for determination of antibody level against NSP of FMD virus has been determined.

Materials and Methods

In the present experiment, one hundred each DIVA positive and negative samples were impregnated to saturation onto pre-cut filter paper strips (Whatman Grade No. 1) of 2.5 cm X 5 cm (Fig-1) size. After absorption, filter paper strips were air dried at room temperature for 4-5 hours and sealed in plastic zip bags (2). In order to evaluate the NSP antibodies to FMD virus, blotted filter paper were kept at 4^0C for future use.

Optimization of sample concentration and size of filter paper : The volume of serum absorbed on the filter paper disc was determined by impregnating a known amount of serum. Randomly, a pre-determined volume of 10 l of serum sample was allowed to be absorbed into the filter paper strip of about 12mm diameter and on measurement, the diameter was found to be 12mm. Then, a filter paper disc that was obtained by using an ordinary paper puncher of diameter about 6mm was used for impregnation of serum samples (Fig.-1). An optimal concentration of 5 ìl serum was found to be absorbed by filter paper disc of 6mm diameter.

Elution of serum antibodies from impregnated filter paper discs : For elution of serum antibodies from the filter paper discs, three types of buffers were used. Two filter paper discs, each of which absorbed 5 l serum were placed simultaneously in 200 l of each of PBS, pH 7.4 (3),

Received : December-2022; Revised : December-2022; Accepted : December-2022

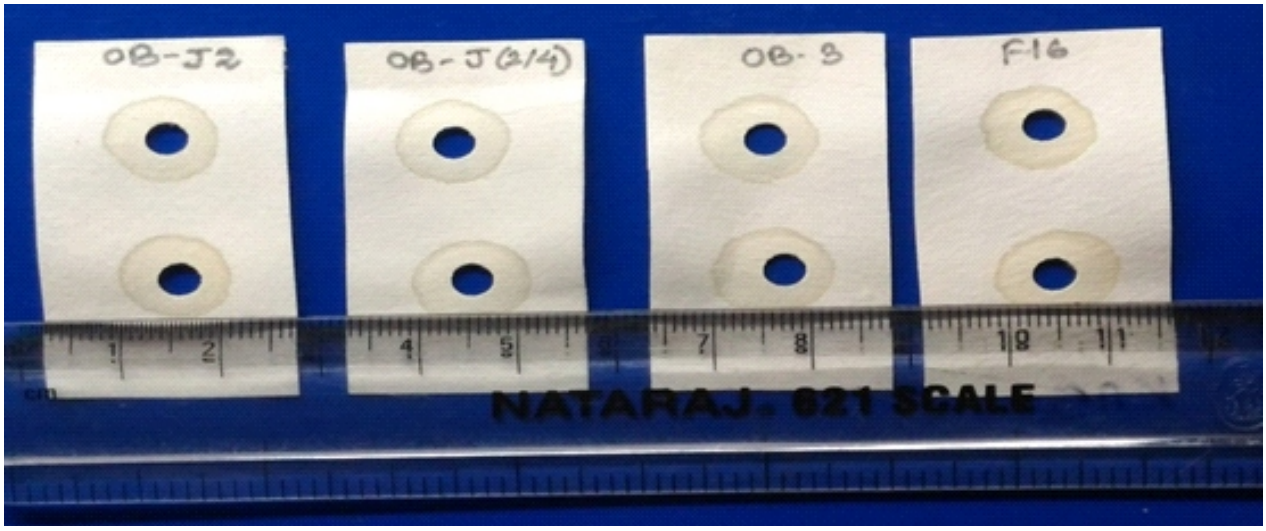

Figure-1 : Serum impregnated filter paper strips from which discs have been cut.

PBS with 0.05 % Tween-20 (2**)** and diluents buffer [r3AB(3) indirect ELISA kit reagent, ICAR-PD FMD, Mukteswar] and incubated at room temperature for 1 hour. Thus, a dilution of 1:20 of serum samples in the buffer used was achieved. Following the elution of serum antibodies, indirect-ELISA was performed using r3AB(3) protein coated ELISA plates as per the protocol recommended by ICAR-PD-FMD, Mukteswar, Uttarakhand. The optical density obtained was then compared for the choice of buffer that could be suitable for elution of antibodies from serum impregnated filter paper discs. Standard serum samples were also tested in the r3AB(3) indirect ELISA and the results obtained were compared with those of samples eluted from filter paper. Sensitivity, specificity, positive and negative predictive values were determined comparing the results of r3AB(3) indirect ELISA between standard serum samples and serum eluted from filter paper were determined using standard method. The sensitivity, specificity and accuracy of the test were determined as per the method described by (4).

True Positive (TP) = the number of cases correctly identified as positive

False Positive (FP) = the number of cases in-correctly identified as positive

True Negative (TN) = the number of cases correctly identified as negative

False Negative (FN) = the number of cases in-correctly identified as negative

$$\text{Sensitivity} = \frac{TP}{TP + FN}$$

$$\text{Specificity} = \frac{TN}{TN + FP}$$

$$\text{Positive Predictive Value} = \frac{TP}{TP + FP}$$

$$\text{Negative Predictive Value} = \frac{TN}{FN + TN}$$

Results and Discussion

In the present study, the diluent buffer supplied by ICAR PD-FMD, Mukteswar along with the r3AB(3) ELISA kit was found to be suitable for elution of serum antibodies from blotted filter paper. Table-1 depicted the sensitivity and specificity of r3AB(3) ELISA using three different buffer. In the present experiment, the sensitivity and specificity was found to be 97% and 100% for blotted serum samples eluted using diluent buffer followed by 92% and 83% using PBS and 94% and 88% using PBST, respectively. The positive predictive value was found to be 100.00, 84.40 and 88.68 % whereas the negative predictive value was found to be 97.09, 92.00 and 93.62 % for blotted serum samples eluted using diluents buffer, PBS and PBST respectively.

In the present experiment, 100 DIVA positive and another 100 DIVA negative serum samples were soaked in pre-cut filter paper. Both the standard raw and blotted serum samples were examined to determine the NSP antibodies to FMD virus. Three different buffers (diluent buffer, PBS and PBST) were used for elution of antibodies from serum blotted filter paper. The sensitivity and specificity of r3AB(3) ELISA was evaluated for detection of FMD virus NSP antibodies in comparison to the standard raw serum samples. The results obtained revealed that eluted samples from blotted serum on filter paper strips perform quite well in comparison with serum samples collected by conventional method. The method has already been successfully applied for the detection of

Table-1 : Sensitivity and specificity of r3AB(3) ELISA for detection of NSP antibodies from blotted serum samples.

Buffer used for elution	True +ve	False +ve	False -ve	True –ve	Sensitivity (%)	Specificity (%)	95% CI (%)		Positive Predictive Value (%)	Negative Predictive Value (%)
							Sensitivity	Specificity		
Diluent Buffer	97	0	3	100	97	100	91.48-99.38	96.38-100	100.00	97.09
PBS (pH 7.4)	92	17	8	83	92	83	84.84-96.48	74.18-89.77	84.40	92.00
PBST	94	12	6	88	94	88	87.40-97.77	79.98-93.64	88.68	93.62

CI- Confidence Interval.

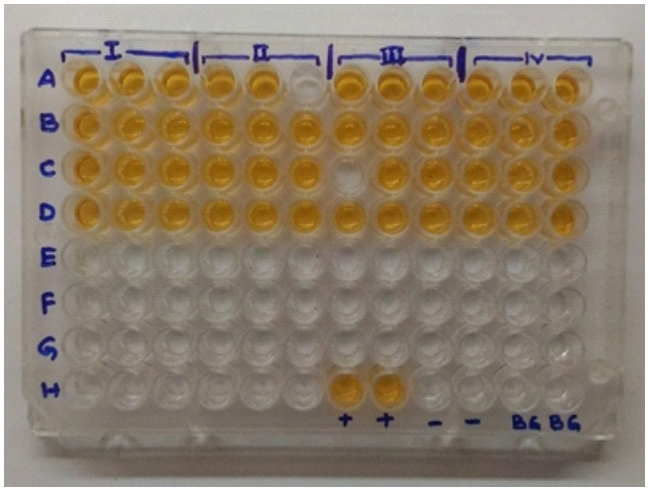

Figure-2 : Photograph showing r3AB(3) indirect ELISA. Row A-D : DIVA positive samples, Row E-H : DIVA negative samples.

antibodies to Chagas disease (5), hepatitis B (6) hepatitis A (7), human immunod eficiency virus (8), Aujesky's disease (9) and Newcastle disease virus (10). In the present study, the diluent buffer supplied by ICAR PD-FMD, Mukteswar along with the r3AB(3) ELISA kit was found to besuitable for elution of serum antibodies from filter paper. Previously, for elution of the serum antibody from blotted filter paper, PBST was used by (2) to determine antibody against bovine herpesvirus type-I without significant loss of sensitivity and specificity. In the present study, r3AB(3) indirect ELISA was used, therefore, diluent buffer comprising *E. coli* lysate was found to be suitable buffer as it may lyse the r3AB(3) coating antigen for binding of specific antibodies. As filter paper is much more convenient for storaging or transporting, the serum sampling on filter paper strip may widely be used for large scale sample screening for differentiating infected and vaccinated animal as well as for evaluation of protective antibody titre for FMD control pragramme.

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