Central Laboratory for Control of
Veterinary Biologics, Abbassia, Cairo, Egypt.

DEVELOPMENT OF INDIRECT SANDWICH ENZYME LINKED IMMUNOSORBENT ASSAY FOR THE DETECTION OF RIFT VALLEY FEVER VIRUS

(With One Table and One Figure)

By
*M.H. ABDEL BAKY and D.M. AL-MUJALLI**
* Dept. of Veterinary Laboratories,
Ministry of Agriculture, Riyadh, Kingdom of Saudi Arabia.

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تطوير اختبار الاليزا الساندوتشي الغير مباشر للكشف عن فيروس حمى الوادي المتصدع

منصور هاشم عبد الباقي ، دخيل بن محمد المجلي

تم تطوير اختبار الاليزا الساندوتشي الغير مباشر للكشف عن فيروس حمى الوادي المتصدع و ذلك باستخدام الأجسام المناعية النوعية المضادة لعتره سميث بيرن والمحضرة تجاريا في الأغنام والفئران. ولقد كان الحد الأدنى لايجابيه الاختبار في الكشف عن الفيروس عند عياريه ٤ Log10 TCID50 /ml.

SUMMARY

An indirect sandwich enzyme linked immunosorbent assay (ELISA) was developed for the detection of Rift valley fever virus using commercial trapping and detecting antibodies (mouse and sheep antisera) raised against Smithburn strain. The marginal positive titer of the virus infectivity detected by the test was 4 $\log_{10}$ $TCID_{50}$/ml (2.7 $\log_{10}$ $TCID_{50}$/50ul).

Key words: *Indirect sandwich ELISA. Rift valley fever, virology.*

INTRODUCTION

Rift valley fever (RVF) virus isolation via inoculation of specimen in laboratory animals or onto cell cultures followed by application of virus neutralization test or hemagglutination inhibition test using reference or standard RVF virus-antiserum is the definite method of diagnosis but it is

expensive and takes time and elaborate work (WHO, 1983). Also, direct detection of RVF virus in tissue specimen by immuno-fluorescence was validated by Swanepoel and Blackburn (1977).

However, ELISA for the detection of RVF virus was developed by Niklasson *et al.* (1983) and Niklasson and Gargan (1985). Such assay is considered the most reliable traditional tool for laboratory diagnosis. Because, there is no available commercial test kit for detection of RVF virus, this study has been taken for the development of a domestic indirect sandwich-ELISA for detection of the virus using commercial mouse and sheep anti-RVF sera.

MATERIALS and METHODS

Virus and culture:

One vial of the commercial live attenuated vaccine of RVF virus, Smithburn strain, batch No. 100 manufactured by Onderstepoort Laboratory, Pretoria, South Africa was used as a seed virus.

Baby Hamster Kidney (BHK) cells were cultured in plastic flasks and grown on Eagle's minimum essential medium (MEM) supplemented with 10% fetal calf serum (FCS) and antibiotics (penicillin sodium 100u/ml, and streptomycin sulphate 100 ug/ml) at 37°C. When the BHK cell cultures became confluent, seed virus of titre 6.5 $\log_{10}$ $TCID_{50}$/vial was diluted 1:500 in MEM and inoculated. After two hours of incubation at 37°C, inoculum was decanted and the culture was supplemented with maintenance medium (MEM containing 2% FCS and antibiotics). After 2-3 days of incubation at 37°C, the inoculated culture fluid as well as uninoculated culture fluid were harvested via two cycles of freezing and thawing followed by centrifugation at 3000 r.p.m for 30 minutes at 10°C and kept at 4°C until titrated and used in indirect sandwich-ELISA.

Titration of virus:

The harvest of BHK cell cultures inoculated with Smithburn strain was titrated in plated BHK cell cultures by a cytopathic effect (CPE) assay. Each 10-fold dilution of the harvest was inoculated onto a group of plated cells in 96-well microtitre tissue culture plate. After 5 days of incubation at 37°C, wells showing CPE were scored, and the titer was expressed as $TCID_{50}$/ml as calculated according to the method of Reed and Muench (1938).

Antisera:

Commercial mouse and sheep anti-RVF sera raised against Smithburn strain by special pathogens unit, National Institute of Virology, Johannesburg, South Africa were tested by serum neutralization test using

a method described by Swanepol *et al.* (1986) for titration of its RVF virus-neutralizing antibodies before its use in indirect sandwich-ELISA.

Indirect sandwich (IS) ELISA:

The test was adopted in guidness of the methods described by Niklasson *et al.* (1953), Niklasson and Gargan (1985) and Roeder and LeBlank Smith (1987).

Polyvinyl 96-well microtitre plate (Nunc, Maxisort plate) was coated with 50ul per well of the mouse anti-RVF serum diluted 1:500 in carbonate. Bicarbonate buffer pH 9.6 and dispensed to 46 wells. After incubation at 4°C overnight, the plate was washed 3 times with 0.002 M. phosphate buffer saline (PBS). 6 successive 10-fold dilutions of the harvest virus of infectivity titre of 6 $\log_{10}$ $TCID_{50}$/ml, as well as the normal (uninoculated) BHK cell antigen were prepared in 0.01 M PBS pH 7.4 containing 0.05% tween 20 and dispensed to the appropriate wells, 4 x 6 wells/each of the virus and normal BHK cells antigen, 4 wells/each dilution, 50ul/well, and the plate was incubated at 37°C for one hour. After washing twice with PBS, sheep anti-RVF serum diluted 1:100 in 0.01 M PBS, pH 7.4 containing 0.05% tween 20 and 5% skimmed milk was dispensed to all the wells of each dilutions of the virus and normal BHK cell antigen (50ul/well) and the plate was incubated at 37°C for 1 hour. Again after washing, a 1:250 dilution of horseradish peroxidase (HRPO) conjugated rabbit anti-sheep (INC, USA) in 0.01 M PBS, pH 7.4 containing 0.05% tween 20 and 5% skimmed milk was added (50ul/well). After one hour incubation at 37°C, the plate was washed and a freshly prepared solution of HRPO substrate (80mg of O-phenyl-enadiamine in 20ml of 0.1 M citrate and 0.2 M PBS plus 0.005% H_2O_2) was added, 50ul to each well. After 20 minutes incubation at room temperature in dark place, 1.25 N H_2SO_4 solution was added (50ul/well), and the absorbance was measured at 492 nm. The average optical density (OD) value for the control normal BHK cell antigen was subtracted from the average OD value of the test virus, corrected values exceeding 0.100 were considered positive.

The test was applied in duplication manner to confirm the obtained results.

RESULTS

Based on the results of RVF virus assay in sheep and mouse anti-RVF sera by serum neutralization test which recorded a titre of 200 and 400, respectively, double fold dilutions 1:250-1:4000 of mouse anti-RVF serum and 1:50-1:400 of sheep anti-RVF serum were chosen in

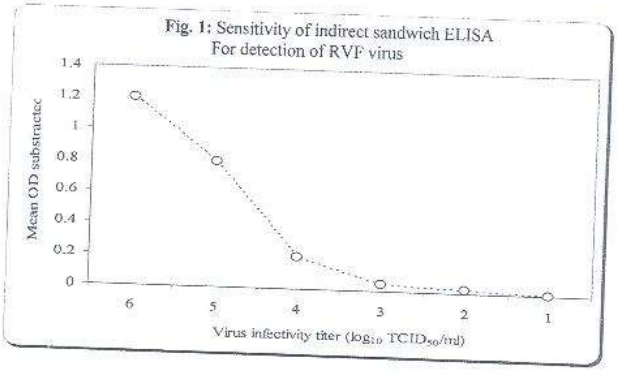
checkerboard titration in the early stage of development of the indirect sandwich (IS) ELISA system for detection of RVF virus in undiluted test virus of titre 6 $\log_{10}$ $TCID_{50}$/ml in parallel to its normal BHK cells antigen. The maximal obtained mean substracted optical density (OD) values range of (1.190-1.208) and (1.170-1.184) were recorded respectively when using dilutions 1:100 and 1:200 of sheep anti-RVF serum against dilutions of mouse anti-RVF serum between 1:250 and 1:1000 (Table 1). The highest non-specific reaction mean OD values were recorded in dilution 1:50 of sheep antiserum against different dilutions of mouse antiserum.

The results summarized in Fig. (1) indicated that the IS-ELISA test proper was capable to detect the virus at infectivity titer of ≥ 4 $\log_{10}$ $TCID_{50}$/ml.

Table 1: Indirect sandwich-ELISA results of checkerboard titration of trapping antibodies (Mouse anti-RVF serum) and detecting antibodies (sheep anti-RVF serum) for detection of Smithburn strain of titer 6.0 $\log_{10}$ $TCID_{50}$/ml

Dilution of sheep anti-RVF serum	Dilution of mouse anti-RVF serum			
	1:250	1:500	1:1000	1:2000
1:50	0.862 *	0.912	0.916	0.788
1:100	1.208	1.202	1.190	0.974
1:200	1.170	1.184	1.176	0.902
1:400	0.746	0.692	0.696	0.652

* Mean OD substracted value (mean OD value of virus – mean OD value of normal BHK cell antigen)



DISCUSSION

Sensitivity of the present indirect sandwich ELISA for the detection of RVF virus using Smithburn strain of known infectivity titre, and trapping and detecting homologous antisera was proved to be useful for monitoring the virus of infectivity titre 6 log_{10} $TCID_{50}$/ml (4.7 log_{10} $TCID_{50}$/50ul) at maximum dilution of 1:100. This means that the test procedure cannot be able to detect the virus of infectivity titre less than 4 log_{10} $TCID_{50}$/ml. This result is nearly agrees with the finding of Niklasson *et al.* (1983) who mentioned that ELISA was capable to detect RVF virus in Rhesus monkeys even with low viraemia (3.5 log_{10} PFU/ml). Commercial test kit of IS-ELISA for detection and typing of foot and mouth disease virus manufactured by reference world laboratory, Pirbright, UK was valid to monitor the virus of infectivity titre $\geq$ 3.8 log_{10} $TCID_{50}$/ml (unpublished data). Unfortunately, validation of the test on field samples was not available, but it could be contributed significantly in detection and/or titration of RVF virus in tissue specimens, blood of RVF-viremic animals as well as in mosquitoes infected with RVF virus.

Nevertheless, the present results demonstrate that the domestic developed IS-ELISA can be used as a rapid diagnostic tool for the diagnosis of RVF and further studies should be applied to achieve the maximum sensitivity of the test if more appropriate reagents and test formulation are selected.

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