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**Title** : **PREPARATION OF ELISA KIT FOR DETECTION OF ANTIBODIES TO BVDV IN CATTLE SERA .**

## **7. ABSTRACT**

In the present study, we prepare ELISA kit for detection of BVDV antibodies in cattle sera. BVDV (NADL strain) was used for preparation of ELISA kit, the virus was passaged in MDBK cell culture and subjected for cytopathogenic and growth behavior studies, then the virus was titrated by using MDBK cell culture. Different methods were used for BVDV concentration for using as virus antigen in ELISA, concentration of the virus using ultracentrifugation, ammonium sulfate and polyethylene glycol (PEG), then the concentrated virus preparations were titrated and the protein content was detected for each one, the results revealed that ultracentrifugation method was the best method for virus concentration and purification to be used as virus antigen in ELISA. Fifty serum samples were collected from vaccinated calves by Cattle Master-4 vaccine and fifty serum samples were collected from non-vaccinated calves, the prepared ELISA kit was used for detection of BVDV antibodies in cattle sera then the results obtained by the prepared ELISA kit were compared with the results obtained by the standard ELISA kit. So we found that the results obtained by the standard ELISA kit were similar to those obtained by the prepared ELISA kit when used the antigen concentrated by ultracentrifugation method.

# List of contents

<i>No.</i>		<i>Page</i>
1.	<u>Introduction</u>	1
2.	<u>Review of Literature</u>	4
2.1	History and distribution of BVDV	4
2.2	BVDV studies in Egypt	9
2.3	BVDV classification	13
2.4	BVDV morphology and physicochemical properties	
16		
2.5	BVDV genome :	17
2.5.1	BVDV genotypes	20
2.5.2	BVDV biotypes (phenotypes)	
23		
2.6	Diagnosis of BVDV :	25
2.6.1	Virus isolation and identification	
25		
2.6.2	Serological diagnosis	
27		
2.7	Immunity of BVDV	37
3.	<u>Material and methods</u>	41
3.1	Propagation of BVDV on tissue culture :	
41		
3.1.1	Material	41
3.1.2	Method	42
3.2	Immunofluorescent antibody technique :	
43		
3.2.1	Material	43
3.2.2	Method	44
3.3	Titration of BVDV (NADL strain) on MDBK cells :	
45		
3.3.1	Material	45
3.3.2	Method	46
3.4	Preparation of the concentrated BVD virus :	
47		
3.4.1	Concentration of the virus using ammonium sulfate	
47		
3.4.1.1	Material	47
3.4.1.2	Method	48
3.4.1.2.1	-Concentration of the virus using ammonium sulfate precipitation	48

3.4.1.2.2	-Dialysis of the concentrated virus against PBS	
48		
3.4.2	Concentration of the virus using PEG	
49		
3.4.2.1	Material	49
3.4.2.2	Method	49
3.4.3	Concentration of the virus using ultracentrifugation	
50		
3.4.3.1	Material	50
3.4.3.2	Method	50
3.5	Titration of the concentrated virus preparations	
51		
3.5.1	Material	51
3.5.2	Method	51
3.6	Determination of protein content of the concentrated virus preparations	51
3.7	Prepared Solid Phase Enzyme Linked Immuno-Sorbent Assay (ELISA)	52
3.7.1	Material	52
3.7.2	Method	54
3.8	Standard Solid Phase Enzyme Linked Immuno-Sorbent Assay (ELISA)	56
3.8.1	Material	56
3.8.2	Method	57
4.	<u>Results</u>	59
4.1	Growth behavior of CP BVD virus (NADL strain) on MDBK cell line	59
4.2	Detection of BVD virus by fluorescent antibody technique (FAT)	60
4.3	Titration of BVDV (NADL strain) on MDBK cell line	61
4.4	Trials for BVDV concentration and purification	61
4.5	Checkerboard ELISA	62
4.6	Results of ELISA using the three prepared concentrated BVDV antigens with vaccinated and non vaccinated serum samples	63
5.	<u>Discussion</u>	68
6.	<u>Summary</u>	75

7.	<u>Abstract</u>	77
8.	<u>References</u>	
78		
9.	<u>Arabic Summary</u>	-
-		
10.	<u>Arabic Abstract</u>	-
-		

## List of abbreviations

5'-UTR	= 5' Un-Translated Region
3'-UTR	= 3' Un-Translated Region
Abs	= Antibodies
AGPT	= Agar Gel Precipitation Test
BDV	= Border Disease Virus
BHV-1	= Bovine Herpes Virus-1
BK	= Bovine Kidney Cells
Bp	= Base pair
BVDV	= Bovine Viral Diarrhea Virus
BVDV-MD	= Bovine Virus Diarrhea-Mucosal Disease
C	= Capsid Structural Protein (P14)
Cp	= Cytopathic strain
CPE	= Cytopathic Effect
CSFV	= Classical Swine Fever Virus
EDTA	= Ethylene Diamine Tetra Acetic acid
EO/gp48	= Enveloped Glycoprotein-O (gp 48)
E1/gp25	= Enveloped Glycoprotein-1 (gp 25)
E2/gp53	= Enveloped Glycoprotein-2 (gp 53)
ELISA	= Enzyme Linked Immuno Sorbent Assay
FBS	= Fetal Bovine Serum
FCS	= Fetal Calf Serum
FITC	= Fluorescence Isothiocyanate
FMD	= Foot and Mouth Disease
Gp	= Glycoprotein/Envelope Surface Protein
IBR	= Infectious Bovine Rhinotracheitis
TFAT	= Immunofluorescent Antibody Technique
IP	= Immunoperoxidase Technique
Kb	= Kilo base
KDa	= Kilo Dalton
MAbs	= Monoclonal Antibodies
MD	= Mucosal Disease
MDBK	= Madin Darby Bovine Kidney Cell Line
MDV	= Mucosal Disease Virus
MEM	= Minimal Essential Medium
NADL	= National Animal Disease Laboratory BVDV strain
Ncp	= Non cytopathic stain
Npro	= Non Structural Protein (P20)
NS	= Non Structural Protein
NS 2-3	= Non Structural Protein 2-3 (P125)
NS 2	= Non Structural Protein 2 (P54)
NS 3	= Non Structural Protein 3 (P80)

NS-4A	= Non Structural Protein 4A (P10)
NS-4B	= Non Structural Protein 4B (P32)
NS-5A	= Non Structural Protein 5A (P58)
NS-5B	= Non Structural Protein 5B (P75)
ORF	= Open Reading Frame
PBS	= Phosphate Buffer Saline
PEG	= Polyethylene glycol
PI	= Persistent Infection or Post Inoculation
PI-3	= Parainfluenza-3
RP	= Rinderpest
rpm	= Revolution per minute
RT-PCR	= Reverse Transcription-Polymerase Chain Reaction
SNT	= Serum Neutralization Test
TC	= Tissue Culture
TCID <sub>50</sub>	= Tissue Culture Infective Dose 50%
VD	= Virus Diarrhea