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Development and Comparison of Capture Enzyme Linked Immunosorbent Assay and Indirect Immunofluorescent Test in Determination of Nairobi Sheep Disease Virus.

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Abstract
The diagnosis of Nairobi Sheep Disease relies on the inoculation of tissue culture, Baby Hamster kidney (BHK-21), with suspensions of infected samples followed by identification of the virus using indirect Immuno-fluorescent assay. These tests have a number of drawbacks including low specificity; visual reading of results which requires highly skilled expertise and tissue culture facilities therefore development of capture Enzyme linked Immuno Sorbent assay (ELISA) would improve the diagnosis of NSD in infected sheep. Nairobi Sheep Disease Virus (NSDV) was isolated, purified and titrated to determine the best working titer for immunization of animals. The purified virus subjected to IIFA test and fluorescence indicated the presence of NSDV. The animals immunized were rabbits and goat which were used for production of antibodies for C-ELISA test. C-ELISA was set-up using anti-goat sera as the primary antibody, purified NSDV as antigen and anti-rabbit sera as the secondary antibody. A 1:400 dilution was established as best dilution for true positive and negative samples. The diagnostic specificity and specificity of the developed C-ELISA was estimated. False positive samples were picked by IIFA, which was confirmed by tissue culture technique. The level of agreement between developed C-ELISA and IIFA used as a gold test was 95%, and the Kappa index was 0.86. The perfect agreement indicated by Kappa values is a sign that both tests can be used. However, C-ELISA is a better test in that it is more flexible and less subjective. The sensitivity and specificity of C-ELISA was estimated at 80% and 100% respectively. The results showed high diagnostic specificity of developed C- ELISA which could be adapted to test a large number of samples over short periods of time. The test is useful during outbreaks of NSD without need for tissue culture facilities.
Keywords: Tissue culture, Capture Enzyme linked Immuno Sorbent assay, Nairobi Sheep Disease Virus, Kappa index.

1. Introduction
Nairobi Sheep Disease is caused by Nairobi sheep disease virus of the genus *Nairovirus* of the *Bunyaviridae* family. It is transmitted by a brown ear tick *Rhipicephalus appendiculatus* (Geering, 1995). NSD has been reported frequently in East Africa and Somalia (Bugyaki, 1957). The disease has been recognized in Mozambique, Congo and Lesotho. In Kenya, NSD occurs in Nairobi, Mount Kenya and Rift Valley areas (Jubb & Kennedy, 1970). The disease was first observed in the vicinity of Nairobi among sheep and goats originating from dry areas in Northern and Southern parts of Kenya (Montgomery, 1917; Dinter and Morein, 1990). NSD causes serious losses to the sheep in the endemic areas and also infected animals can spread NSD to non-endemic areas (Fenner *et al*., 1987). The current diagnostic test used in detection of NSDV is indirect Immunofluorescent assay (IIFA). However IIFA requires tissue culture techniques which risks contamination and growing of cell lines which take time. The interpretation of IIFA results requires visual scoring which is subjected to errors and. only a few samples can be analysed at any given time. There was a need for an alternative technique. Capture ELISA was tested as alternative.

2. Materials and Methods
The study was carried out at Biotechnology centre of Kenya Agricultural Research Institute (KARI). Known sample of NSD-G202 isolated from a sheep from Laikipia was used for the development of capture Enzyme linked Immuno Sorbent assay (ELISA). The virus was multiplied in baby hamster kidney-21 cell at 37^{o}C (OIE, 1996). The virus was harvested after 3-5 days after the cytopathic effect were formed and then frozen at - 20 ^{o}C and Cryopreserved until use.
The virus was purified and prepared by use of sucrose gradient method (Terpstra, 1983). The purified G-202 NSD was titrated and serially diluted in 96 well plates to obtain the working titre for the immunization of animals. The titer of the virus was determined as ($TCID_{50}$ virus titre). The animals were injected with the purified NSDV subcutaneously at several sites with 200ul of purified NSDV per site as described by Harlow and Lane (1988). After boosting the animals with the virus the blood was collected and serum prepared. Capture ELISA

was set as described by Goldsby et al., (2003) with little modification at different dilutions. Cryo-preserved samples were tested using the developed C-ELISA and indirect Immunofluorescent test, the latter being the gold standard test. The data was determine by Kappa test and sensitivity and specificity of the C-ELISA was calculated using contingency table (Martin et al., 1987)

3. Results

The working titer for the immunization of animals was at 1:320. The immune serum from goat and rabbit when tested using indirect Immunofluorescent test to confirm NSDV strong fluoresces was observed at 1/10. Titration for C-ELISA showed the titer of rabbit and goat antisera at 1/128 concurrently with the optical density of 490 as shown on Figure 1 and 2.

Twenty samples were tested for NSD using the two tests. Four samples (20%) were positive with C-ELISA and five samples (25%) positive with IIFA as shown on (Table 2). The IIFA was used as gold test because it was the test currently in use. The results are illustrated in figure 3. The agreement between C-ELISA and IIFA was 95% and the kappa index was 0.86 giving perfect agreement. The sensitivity and specificity of C-ELISA was calculated against the gold standard test in the formula given. The specificity of C-ELISA was 100% while the sensitivity was 80%.

4. Discussion

This study involved development of C – ELISA to be used for detecting NSDV. The current test IIFA was used as a gold test. The IIFA requires tissue culture techniques and interpretation of results requires more than one reader and requires experienced personnel to read (Desselberger, 1995). Out of the twenty samples tested, four (20%) were positive for C-ELISA and five (25%) were positive for IIFA. The four positive samples from the both tests were from the same sample except that one sample which was positive for IIFA as indicated in the results. All the samples which were positive were subjected to tissue culture technique which is normally the confirmatory test. This was an indication that one positive sample from IIFA was false positive. The BHK - 21cell lines are specific for NSDV if the CPE was not observed then it meant absence of the virus. There was clear sign that IIFA test can pick false positive samples. The diagnostic specificity and sensitivity of C- ELISA was 100% and 80% respectively as indicated in the results. The small number of samples could have contributed to low sensitivity. From other studies when comparison between ELISA and IIFA was done the observation indicated that the diagnostic sensitivity of ELISA was between 90 – 84% and specificity was 96.6 – 100% (Araulo et al., 1998). The sample size in such studies was large. The reasons for using small sample size were due the fact that there was no outbreak of NSD and cost constraints. The diagnostic specificity and sensitivity of IIFA from other studies were 75.2% and 79.5% respectively (Pare et al., 1995). This indicates that IIFA is hindered by subjective variation of results due to the personnel who might be blinded by the ultra violet microscope. From this observation it was concluded that ELISA is a simple, sensitive and highly specific method.

5. Conclusion

There was an agreement between C-ELISA and IIFA as indicated by Kappa index of 0.86 this is indication that the two tests can be used but IIFA is very subjective and requirement of tissue culture facilitate which is very laborious.

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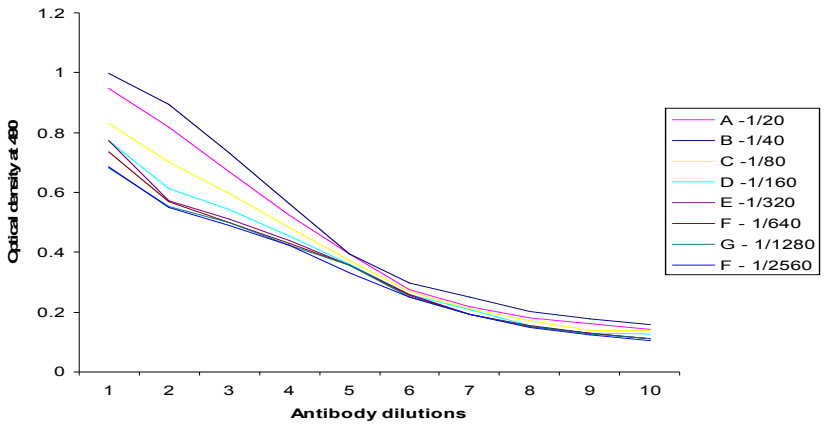

Figure 1: OD readings of different dilutions of NSDV and each reacted with varying dilutions of rabbit antisera.

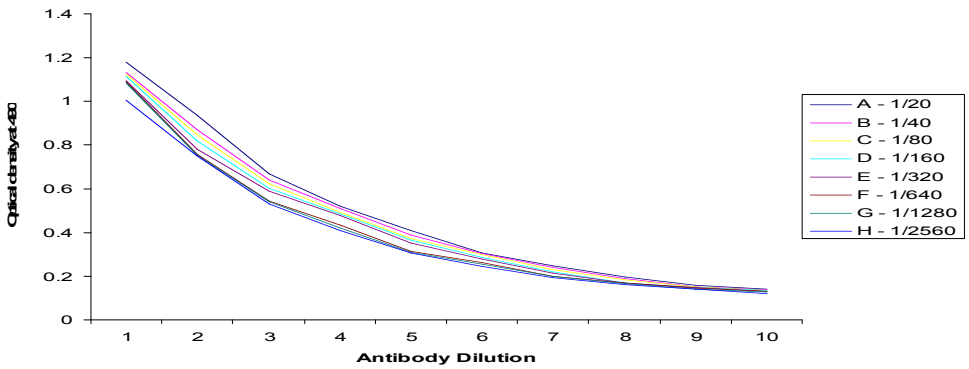

Figure 2: OD readings of different dilutions of NSDV each reacted with varying dilutions of goat antisera.

Table 1: Calculated against the gold standard test (IIFA)

		Positive	Negative	Total
C-ELISA	Positive	a	b	a+b
	Negative	c	d	c+d
	Total	a+c	b+d	a+b+c+d

(Adapted from Martin *et al.,* 1987)

Sensitivity (%) = a * 100 / (a+c)
Specificity (%) = d * 100 / (b+d)
Predictive value (%) of negative results = d * 100 / (c+d)
Predictive value (%) of positive result = a * 100 / (a+b)
False negative rate = I – Sensitivity
False positive rate = I - Specificity
% Concordance = proportion a+b or ((a+d)/ (a+b+c+d))* 100
Kappa index = (OP-EP)/ 1-EP where;
EP = Expected Agreement = ((a+b)/ n* (a+c)/n)) + ((c+d)/n*(b+d)/n))
OP = observed agreement (Concordance)

The scale used for evaluating kappa values was as follows:-

Kappa: 0 - 0.2, 0.21 – 0.40, 0.41 – 0.60 above 0.81
Agreement slight fair moderate perfect
(Adopted from Smith, 1995)

Table 2: Comparison of Capture Enzyme Linked Immuno Sorbent Assay and Indirect Immunofluorescent Test

IIFA				
		Positive	**Negative**	**Total**
C-ELISA	**Positive**	**4**	**0**	**4**
	Negative	**1**	**15**	**16**
	Total	**5**	**15**	**20**

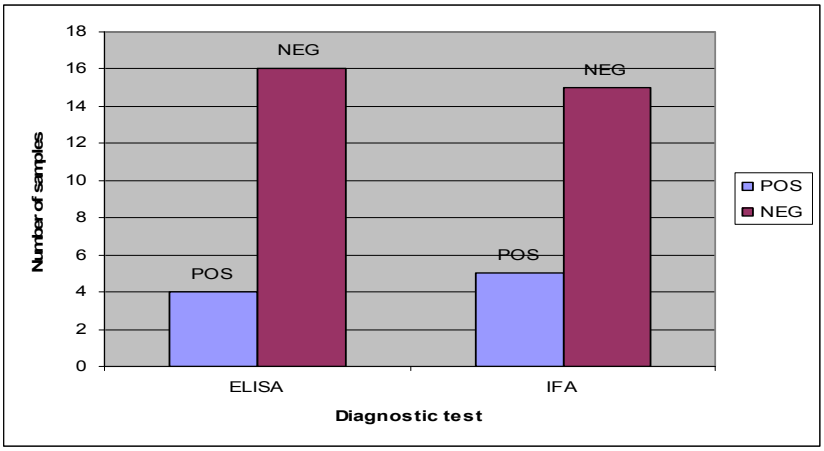

Figure 3: Positive and Negative samples from the two tests

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