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FINAL REPORT

DLS
ABUKO
20-12-1990

1228

TRAINING OF 2 (TWO) LABORATORY TECHNICIANS IN THE USE OF THE INDIRECT ELISA TECHNIC FOR RINDERPEST SERO-SURVEILLANCE (PAN AFRICAN RINDERPEST CAMPAIN) AT CENTRAL VETERINARY LABORATORY, ABUKO : FOLLOW-UP TO TRAINING COURSE AT LNERV DAKAR-HANN, REPUBLIC OF SENEGAL

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Dr. J. SARR

Terms of reference

1 - To train 2 Gambian Laboratory Technicians in the use of Rinderpest ELISA Technic (Central Research Laboratory Dakar-Hann, Senegal).

2 - To visit Gambia (5-24 december) as a follow-up to the Rinderpest ELISA training courses at LNERV Dakar-Hann, in order to assist DLS and other researchers establish the ELISA test and carry out quality control and test runs with the two laboratory technicians trained at LNERV.

3 - To determine a negative OD cut-off value from Gambian negative cattle population to be compared with the senegalese negative OD cut-off value.

4 - To make recommandations in respect of the Gambia Rinderpest ELISA based on quality control findings.

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Counterparts

- Dr E. M. TOURAY - Head, Research and Investigation DLS
- Dr Badara LOUM - (Directorate DLS)
- Dr Jarra JAGNE - Research and Investigation DLS / ABUKO Clinic
- Dr Roland MINOR - EEC/PARC Technical Adviser.

Participants

- Mr Biram FYE - Trained at LNERV
- Ms Tida BOJANG - Trained at LNERV
- Ms Fatou CEESAY - Animal Husbandry Officer
- Ms Fatou DABOR - Trainee Livestock assistant
- Ms Lissa FYE - Laboratory attendant
- Ms Ida NYASSI - Trainee Livestock assistant
- Ms Danetta MBYE - Laboratory attendant.

Introduction

Gambia has no focus of Rinderpest since 1965. After the first rinderpest panafrican campain JP 15, Gambia used to vaccinate against rinderpest every year until 1987.

Hence, in its rinderpest eradication program, the Gambia will commence with step 2 and 3 of the 3 steps of the eradication of, and providing freedom from rinderpest infection detailled in the report of the Expert consultation in Rinderpest Surveillance (OIE 1989).

1 - rinderpest control,

2 - rinderpest clinical surveillance,

3 - rinderpest serological surveillance.

The rinderpest ELISA technic will be used in all countries participating in the PARC Rinderpest Serological Surveillance (Guidelines for seromonitoring of cattle conducted by PARC, OUA/IBAR/PARC oct. 1988).

Hence, two laboratory technicians had been trained for one month in the use of ELISA at Laboratoire National de l'Elevage et de Recherches vétérinaires of Dakar-Hann (LNERV), to enable them to participate effectively in the PARC Serosurveillance program :

- Mr Biram FYE 1 - 30 sept. 1990,
- Ms Tida BOJANG 1 - 30 sept. 1990.

The main objective of my consultancy (5 - 24 december 1990) as a follow-up to the rinderpest ELISA training courses at the LNERV of Dakar Hann, is in order, to assist the Department of Livestock Services (DLS) of the Gambia and other researchers to establish the ELISA test and to carry out quality control and test runs with the Laboratory technicians (trained at LNERV) within Abuko Veterinary local conditions.

The ELISA training program was funded by the GARD project (USAID).

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Quality control I

With LNERV distilled water (Dakar-Hann) :

different serum dilutions both from the positive and the negative references were used.

Table 1

	Serum dilutions 1/4 1/8 1/16 1/32 1/64 1/128
Blank	Positive reference serum
Blank	Negative reference serum

The antigen was diluted at 1/100e in PBS and serum samples in blocking buffer (PBS + 5 p.100 skimed milk).

Results

Table 2

Serum dilutions		1/4	1/8	1/16	1/32	1/64	1/128	Blank
OD values								
Positive Serum	1	0.599	0.460	0.388	0.280	0.207	0.184	0.032
	2	0.551	0.420	0.402	0.310	0.166	0.192	0.024
Mean value		0.575	0.440	0.395	0.295	0.186	0.188	0.028
OD values								
Negative Serum	1	0.047	0.044	0.039	0.029	0.035	0.023	
	2	0.053	0.037	0.022	0.031	0.028	0.030	
Mean value		0.050	0.040	0.030	0.030	0.031	0.026	

OD = Optical Density

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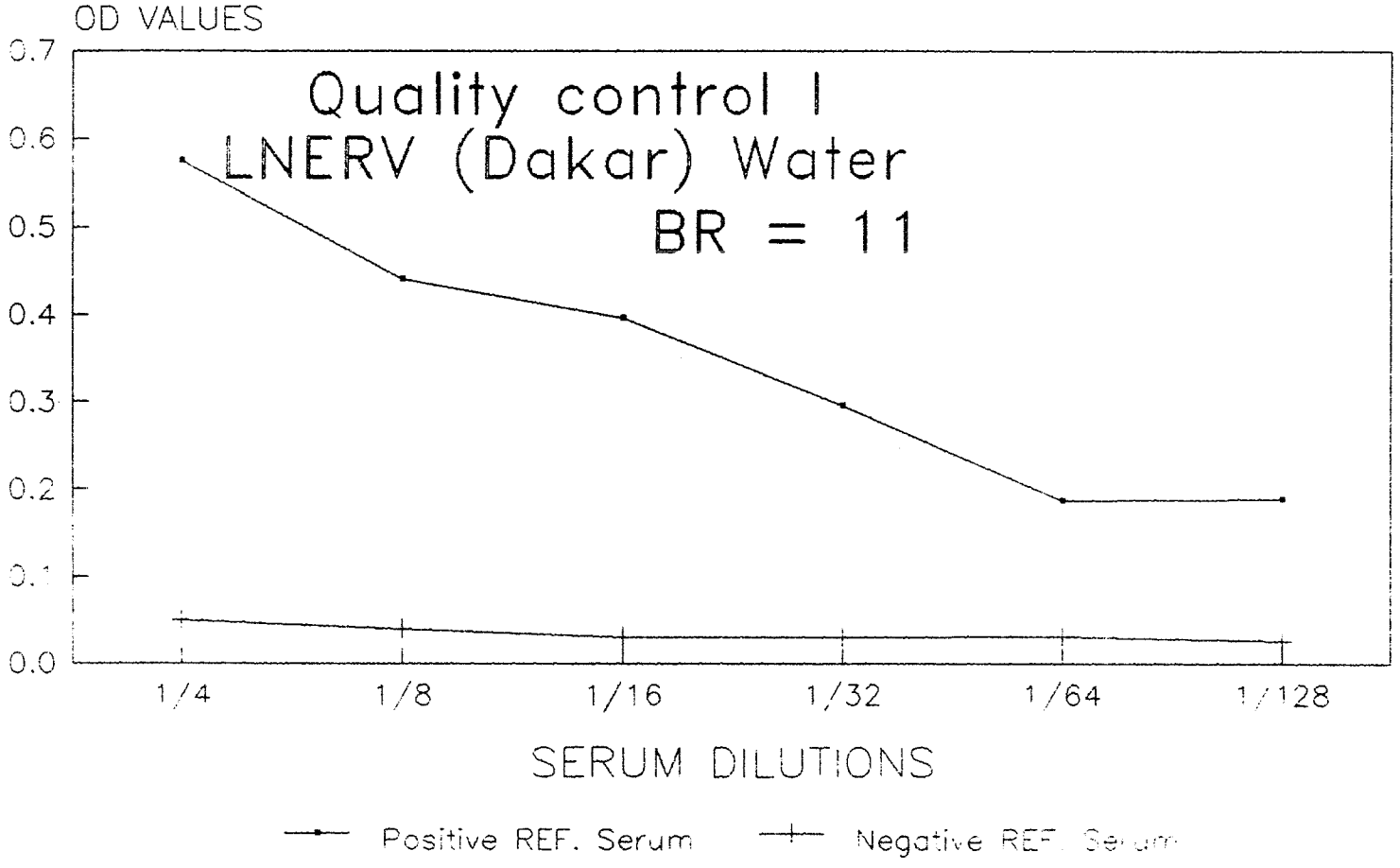
Quality control I

Rinderpest sero-surveillance



ELISA

The interval between the positive reference and the negative reference sera was higher at a dilution of 1/4e.

The corresponding binding ratio, BR = 11 (see graph.).

Conclusion

The ELISA test proved to be workable at the under Abuko Veterinary Laboratory conditions.

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Determination of the Gambian negative OD cut-off value for the rinderpest ELISA

148 serum samples were been taken in the lower Niumi District-North bank Division from 1 - 2 years old animals.

The last rinderpest vaccination campain was held in 1987.

All serums samples have been taken randomly in different herds.

Tests were conducted with ITC deionized water.

Results are summarized in histograms 1, 2.

Plate I

BR = 5

$\overline{X}$ = 0,045

$2\overline{X}$ = 0,090

From plate I, only one serum sample can be considered to be positive.

Plate II

BR = 4,4

$\overline{X}$ = 0,048

$2\overline{X}$ = 0,096

All the serum samples were negative.

Conclusion

In the Niumi-District-North bank Division, there is no strain of rinderpest virus in circulation among cattle from 1987 up to now.

The serum samples with an OD value equal or less them the mean OD of the negative reference were pooled for the determination of the national negative reference.

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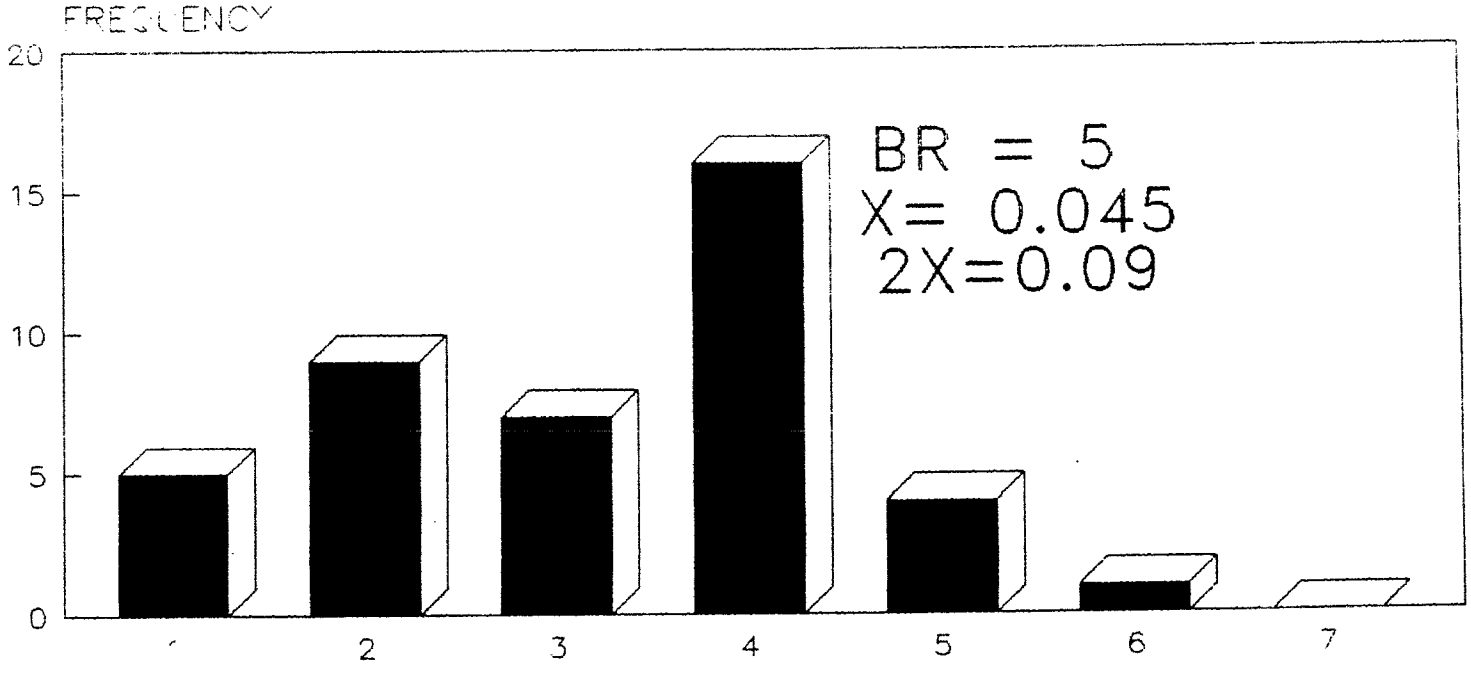
Histogram 1

National Negative Plate 1

ITC Water



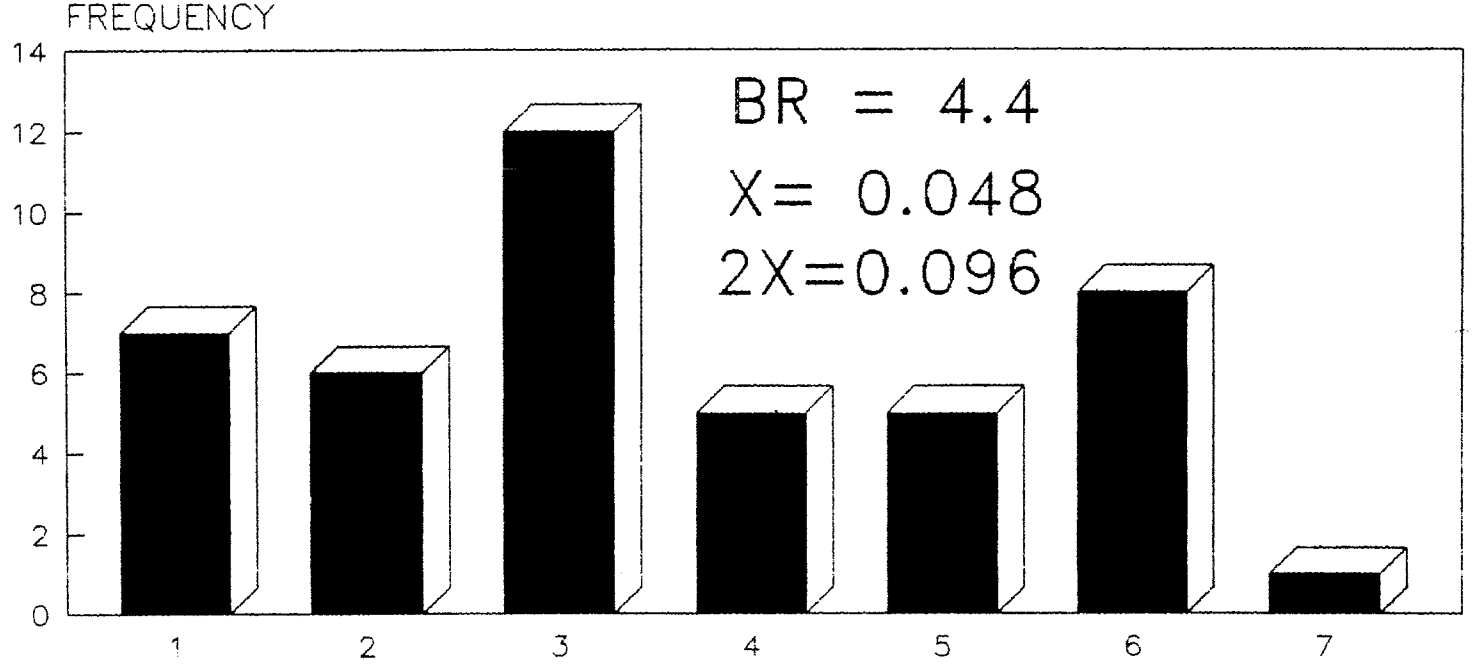
1=0.04–0.05 2=0.051–0.06 3=0.061–0.07
4=0.071–0.08 5=0.081–0.09 6=0.091–0.1

ELISA

Histogram II

National Negative Plate II

ITC Water



1=0.02–0.03 2=0.031–0.04 3=0.041–0.05

4=0.051–0.06 5=0.061–0.07 6=0.071–0.8 7=0.081–0.09

ELISA

Quality control II

As the national negative reference serum was compared to the positive reference serum from the AIEA kit using ITC (International Trypanotolerance Center) deionized water.

Serum Dilution	1/4	1/8	1/16	1/32	1/64	1/128
Positive Ref.	0.261	0.251	0.189	0.115	0.129	0.090
	0.272	0.196	0.173	0.111	0.144	0.077
Mean OD	0.266	0.223	0.181	0.131	0.136	0.083
Gambia National negative control	0.048	0.036	0.026	0.013	0.07	0.010
	0.057	0.031	0.016	0.019	0.006	0.009
Mean OD	0.052	0.033	0.021	0.016	0.006	0.009

At a dilution rate of 1/4 of the positive (Kit) and the Gambian national negative : BR = 5 The binding ratio was found to be 5.

$$\bar{X} = 0.052$$

$$2\bar{X} = 0.104$$

Thus there was no significant difference in the binding ratio (BR) of the kit and the national reference positive over negative (BR).

Conclusion

The pooled serum from which the national negative was derived could be stored in the serum bank as a source of a national negative control.

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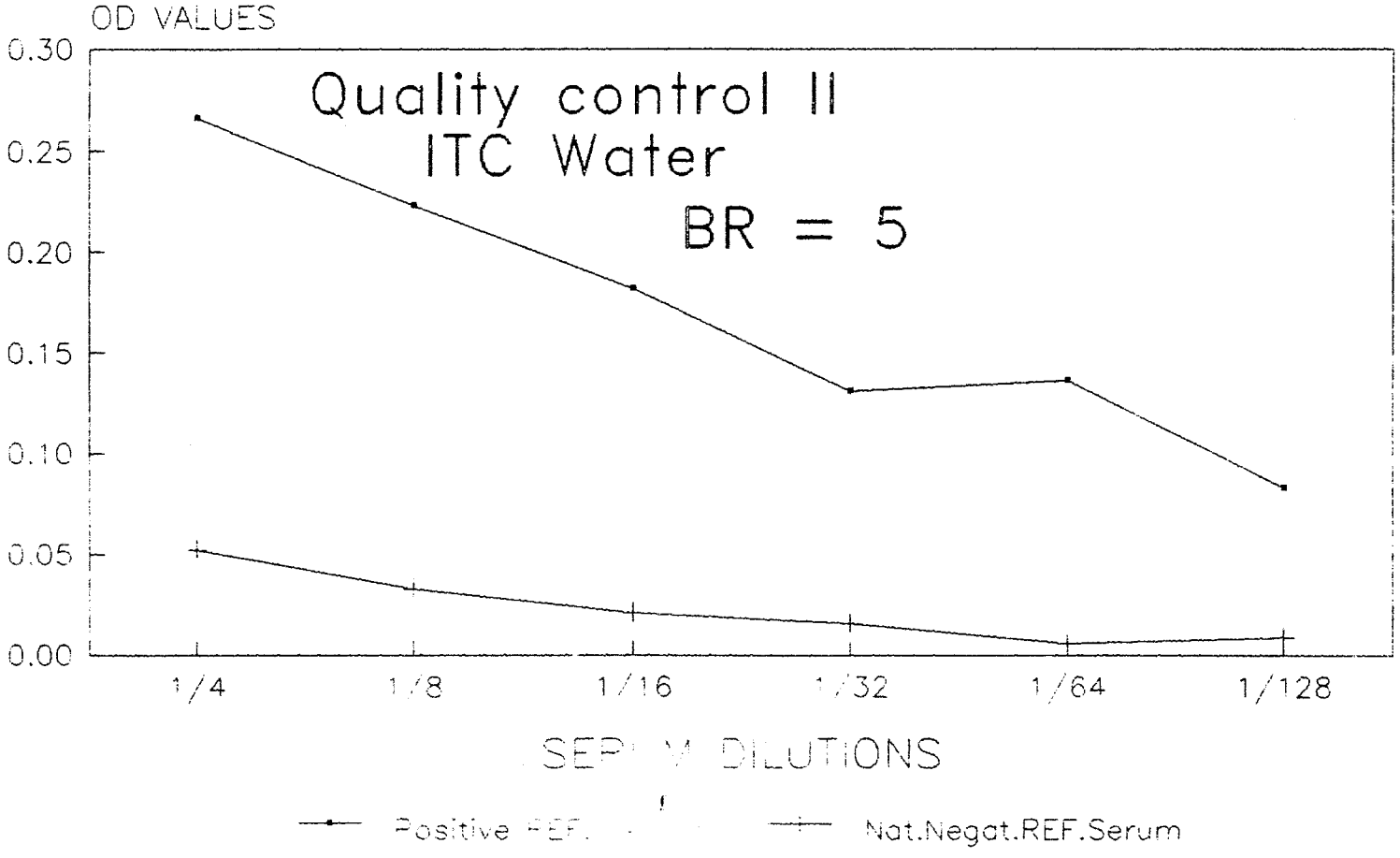
Quality control II

Rinderpest sero-surveillance



ELISA

General conclusion

The rinderpest ELISA test can be run in Abuko Veterinary Laboratory under local conditions.

However, the binding ratio (positive reference/negative reference, or national positive reference / national negative control) could be higher than was determined in our experiments.

It depends essentially on the water quality used in the test. The ITC (International Trypanotolerance Center) deionizedwater could be used because it gives relatively good results.

The Veterinary Laboratory of Dakar-Hann (LNERV) will assist the Abuko Veterinary Laboratory by supplying bi-distilled water for the AIEA Kit reconstitution reagents. The ELISA test can be a very usefull technic not only for rinderpest sero-surveillance but also for the seromonitoring of other diseases in the Gambia.

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Recommandations

1 - Check water quality control

2 - Check the ph of all solutions before starting the test : PBS, washing buffer, blocking buffer...

3 - OPD buffer should be acid
If OPD buffer is not available, distilled water can be used.

4 - Carefull pipetting (with the multichanel pipette) will avoid significant differences between values obtained at the same dilution.

5 - Plate washing should be done properly to avoid non specific fixation of serum proteins or conjugate.

6 - One technician should be trained in the use of computers.

7 - Technical assistance for at least 2 weeks will be necessary for the starting of rinderpest seromonitoring test when all equipment and serum samples are available in the laboratory.

8 - IAEA should assist Dr E. M. TOURAY for analysing data for the final report (1 epidemiologist 2-3 weeks).

9 - Through Dr E. M. TOURAY's IAEA research contract, request for the rinderpest ELISA from IAEA, Vienna, Austria.

10 - It will be necessary to maintain liaisons between DLS and LNERV in order to resolve problems encountered in the running of the ELISA test (Quality control).

11 - The Laboratory team should be involved in the field serum sampling for sero-monitoring : sampling should be carried out by laboratory staff as a separate team.

12 - For the Gambia rinderpest sero-monitoring program, 2 000 serum samples are enough considering the cattle population of 350 000 and a probability of 95 % confidence.

13 - I would like to suggest Dr E. M. TOURAY to attend a 3 days seminar at LNERV (Dakar) 15 - 17 january on the fundings at rinderpest ELISA training follow-up.

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Equipments to be ordered

- 10 000 tips
- 20 Ks (troughs)
- 40 micronic tube holders
- 5 000 micronic tubes
- 50x 5 box microplates for ELISA
- 1 multichanel pipette 5-50 fl.
- 1 single chanel pipette 5-50 fl.
- 1 single chanel pipette 50-200 fl.
- 1 plate shaker
- 1 water container 20 l
- 1 water container 50 l
- 1 computer unit
- 1 ph-meter
- 1 ELISA reader (multiskan MK II Plus)
- 1 distilled water unit
- 1 refrigerator (high capacity)
- 1 deep freezer (serum bank)
- 1 bacteriological incubator
- 1 sensitive balance
- sampling material (vacutainers, needles, etc...)

Aknowledgments

Gratiful thanks to DLS, and the GARD project for financial and logistical support,

Dr E. M. TOURAY and all the technicians at the Central Veterinary Laboratory for their valuable contributions to the success of the follow-up Rinderpest ELISA training course at Abuko.