PROJECT 3P-82-0225-03

YELLOW FEVER

INTERNATIONAL DEVELOPMENT RESEARCH CENTER - CANADÁ

FUNDAÇÃO OSWALDO CRUZ - BIO-MANGUINHOS - BRASIL

FINAL REPORT

INTRODUCTION

This report covers activities developed by Oswaldo Cruz Foundation/Bio-Manguinhos during the period from August 1984 to May 1985, end of the project 3P-82-0225-03, Yellow Fever (Latin America), International Development Research Centre, Canada. It is divided in three main lines as described in the Interim Report:

1. Stabilized vaccine: Among candidate stabilizer substances described in the Interim Report were chosen sucrose and sodium glutamate. Both substances were added to the embryo juice before lyophilization and results obtained have justified the use of an improved formulation for Yellow Fever vaccine.

2. Studies on stability of reconstituted vaccine were continued aiming to find a candidade diluent which would maintain potency of diluted vaccine;

3. Aspects regarding Yellow Fever vaccine production, aiming to standardize its quality control;

RESULTS

Methods used during these studies were basically those described in the Interim Report. Virus titrations were performed using Vero cells and a reference vaccine was included in all tests.

1. Studies with stabilized vaccine

In this report "standard" means the vaccine produced according to methods described by Penna (1), that is, a pure supernate of centrifuged embryos without any substances added to it. "Stabilized" refers to vaccine prrepared with stabilizers which formulation will be described below.

In the Interim Report it was shown that several substances as sugars (sucrose, sorbitol) and aminoacids (arginine, glycine, sodium glutamate) when added to final bulk of yellow fever vaccine showed a protective effect on final product thermostability.

In addition, it was also shown that a reasonable quantity of 17D virus could be extracted from the sediment of the centrifuged production bottles with 199 medium and that extra virus could be used to increase the volume of produced vaccine.

According to these data, it was tried a new formulation for yellow fever vaccine. Sucrose and sodium glutamate were chosen as stabilizers due to their availability in local market and lower price. To form the final bulk, they were added to a mixture of embryo juice and resuspension of the sediment in the following proportion: embryo juice 50%, resuspension 30% and

stabilizers 20%. This new, stabilized vaccine gave an economy of 50% over the standard vaccine which utilized pure embryo juice.

Twelve lots of stabilized vaccine were prepared and control tests of final product.

1.1. Physical, chemical and biological characteristics

All control tests recommended by WHO (2) were performed and all 12 lots approved. It is important to notice that protein nitrogen content was not over 0,25 mg per human dose (maximum limit required by WHO) in spite of sodium glutamate and 199 Medium utilization. Data are shown in table 5.

1.2. Losses during lyophilization cycles

Losses observed in virus titre during lyophilization cycles were lower with the stabilized vaccines suggesting that stabilizers added to final bulk also have presented a protective effect on 17D virus during that operation (table 2).

1.3. Thermostability

All 12 lots of stabilized vaccine were exposed at 37C durint two weeks and they lost in average 0,35 log of virus titre. This finding is in contrast with those obtained with standard vaccine which lost 1.92 log in averaga (table 4).

As recommended by WHO (2) a vaccine to pass thermostability test should lose no more than one log after two weeks of exposure at 37C. Following this criterion, all 12 lots of stabilized vaccine comply with the test while from 23 lots of standard vaccine only two (8.7%) would pass it. Another point is that there is an increase in standard deviation (from 0,25 to 0,63) and coefficient of variation (from 4.38 to 16.9%) of the exposed standard vaccine while for the stabilized product, both standard deviationand coefficient of variation maintained the same level, suggesting that degradation phenomema which ocurrs at 37C are less variable with the stabilized vaccine. Data are shown in table 4.

1.4. Titre of final product

All 12 lots of the stabilized vaccine were over the limit of 3 log per human dose as required by WHO (2). However, the titre of the stabilized lots were lower than the standard vaccines (table 3) probably due to the dilution of embryo juice.

2. Studies with reconstituted vaccine

A number of experiments were performed to continue the studies on 17D virus stability in reconstituted vaccine. Vials of standard and stabilized vaccines were reconstituted and diluted with various diluents as recommended to contain 3 to 4 log of virus per human dose.

Table 6 shows results obtained with several candidates as diluents and it can be seen that distilled water presented a higher capacity for 17D virus protection, maintaining its titre up to 6 hours of incubation at 37C. This finding was unexpected and deserved to be further explored. Table 7 presents data showing that 17D virus stability in distilled water is comparable to that given by bovine serum. Table 8 shows that distilled water is a good diluent for both standard and stabilized vaccines and table 9 and figure 3 show that several solutions presented poorer results than distilled water and that actually standard vaccine increased its titre.

3. Vaccine production quality control

3.1. Titration of virus in production bottles: Table 1 shows results obtained during routine titration of 17D virus in bottles containing triturated chick embryos. Forty three harvests in a total of 1.087 bottles, comprehending about 50.000 eggs were examined. It can be seen that the embryo juice average titre is 5.94 log per ml, with a standard deviation of 0.30. This results is similar to that obtained last year.

3.2. Titre loss during lyophilization cycles

To determine how much virus was being lost during a lyophilization cycle, samples of final bulk were titrated and compared with final product titre. Table 2 shows results obtained with lots of standard and stabilized vaccines and it can be seen that the standard vaccine loses an average of 0.52 log during a lyophilization cycle where as the stabilized one loses 0.31 log.

3.3. Titre of final product

Table 3 shows titres of final product and it can be seen that those obtained with standard vaccine were higher than the stabilized one. All produced lots had titers over the limit established by WHO of 3 logs per human dose (2).

3.4. Thermostability

Vaccine thermostability tests were performed according to the method recommended by WHO (2). Vials of dried vaccine were exposed during two weeks at 37C and titrated using flasks of the same lot kept at -20C as controls. Results are shown in table 4 and figure 1 and it can be seen that standard vaccines lose 1.55 log in average after two weeks of exposure at 37C, being this results similar to that shown in the Interim Report. On the other hand, stabilized vaccines lose 0.35 log in average.

3.5. Identity

All produced lots were approved in this test (data not shown).

COMMENTS

Yellow Fever vaccine as it was being produced by Bio-Manguinhos/Oswaldo Cruz Foundation, in spite of several technological improvements, followed techniques described in 1954 by Penna (1).

The standard vaccine, which complied with requirements made by WHO(2), presented a low thermostability, which shortened its shelf life and was unstable after reconstitution with saline. Thermostability results presented in Table 4 showed that standard vaccine lost 1.95 log of its titer after exposure at 37C during two weeks as recommended by WHO (2). Actually, from 23 standard vaccine lots studied, only 2 (8.7%) would be approved in this test. Moreover, reconstituted standard vaccine, in spite of maintaining its titer when kept in ice-bath, presented a rapid inactivation when exposed at 37C. These characteristics, that is, a low thermostability of the dry product and the instability of reconstituted vaccine should be modified due to the fact that this vaccine is used in rural areas where the cold chain is not always reliable and vaccinacion is usually performed under high ambient temperatures.

By these reasons, tha main objectives of this project were to improve Yellow Fever vaccine in both aspects. In order to achieve them, it was necessary to standardize 17D virus titration in Vero cells and to maintain a continuous quality control process for vaccine production.

Aspects and preliminary results were reported in the Interim Report and have continued to be done during the present period. Following this line of work, it was determined virus content in infected embryo juice, (table 1), losses that occurred during a lyophilization cycle (table 2) and thermostability (table 4). Other parameters as potency (table 3) and identity were also determined and it can be seen that standard vaccine complies with WHO requirements for Yellow Fever vaccine (2), with the exception of thermostability.

The second part of this study aimed to find a suitable diluent to reconstitute and dilute Yellow Fever vaccine. Since the beginning of Yellow Fever vaccine production (1), the recommended diluent was Ø,15 M saline, which is being used for almost all production laboratories.

Some tests reported in the Interim Report showed that vaccines reconstituted with saline were able to maintain their virus titre only if held in ice both while those incubated at 37C lost their titre in less than one hour.

It was found that vaccine reconstituted and diluted with distilled water would maintain the virus titre during incubation at 37C for at least 6 hours (tables 6, 7 and 8) and this phenomenom occurred with bath stabilized or standard vaccines. In the literature only one reference was found dealing with the

stability of Yellow Fever virus suspended in distilled water (3). It was a work done in 1930 with no conclusive results on the action of this substance when used to dilute rhesus monkey serum or mosquitoes containing Yellow Fever virus to be filtered through Berkfeld candles.

Yellow Fever vaccine reconstituted and diluted with distilled water retained its titer for at least 6 hours incubation at 37C (table 6) and it was shown that the titre of the virus was similar to others diluents containing serum (table 7) and these results were observed with both types of vaccine (table 8). It can be concluded that Yellow Fever vaccine can be reconstituted and diluted with distilled water and will be able to retain its titer when exposed at 37C for 6 hours at least. The reasons why distilled water is the best diluent for Yellow Fever vaccine produced at Oswaldo Cruz Foundation remain unknow up to this moment.

Concerning vaccine stability, several substances have been added to the final bulk before lyophilization aiming at an improvement of its thermostability. Besides, it was also found that a significative amount of 17D virus could be recovered from the sediment of bottles centrifuged to obtain embryo juice. The protective action of those substances and virus extraction from sediment were described in Interim Report.

Among substances candidate as stabilizers, it was chosen sucrose and sodium glutamate, due to their availability and price. To prepare stabilized vaccines, sucrose and sodium glutamate and a resuspension of sediment were added to embryo juice in proportions already described.

Twelve lots of this vaccine were prepared and obtained results showed that their chemical, physical and biological characteristics complayed with requirements issued by WHO (2) and data are shown in tables 3 and 5. Moreover, the thermostability of this stabilized vaccine was better than that observed with standard vaccine as all 12 prepared lots lost less than 0,5 log of virus titre after exposure at 37C (table 4).

In conclusion, the three main lines of project 3P-82-0225-03 were accomplished with success. A titration method for 17D virus using Vero cells was standardized and it is being used to control production and final product, a new improved yellow fever vaccine with a good thermostability is being produced and a suitable diluent was found. All these problems had to be solved due to the characteristics of Yellow Fever vaccination which is utilized in rural areas of tropical countries where the cold chain is not reliable and vaccination is performed under high ambient temperatures. Besides, this new formulation allows an economy of 50% on the use of infected embryos, giving an increase of the production that combined with an enlargement of its shelf life allows to build up a strategic stock to face epidemics in rural or urban areas.

REFERENCES

1. Penna, H.A. WHO Monograph Series n. 30, 1954 pp. 67-90.

2. WHO Technical Report Series n. 594, 1976.

3. Bauer, J.H. and Mahaffi, A.f. - Am. J. Hyg. 12: 175-195, 1930.

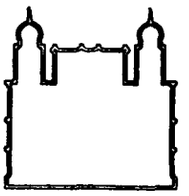
TABLE 1

YELLOW FEVER VACCINE. TITRATION OF BOTTLES CONTAINING EMBRYO PULP

PROD #	DATE	QUANTITY OF BOTTLES	AVERAGE TITRE	STANDARD DEVIATION	COEFFICIENT OF VARIABILITY
29	04-06-84	20	6.05	0,23	3.80
30	11-06-84	20	5.11	0,16	3.08
32	14-06-84	21	6.22	0,16	2.51
34	25-06-84	30	6.21	0,25	4.13
35	28-06-84	28	6.13	0,18	3.04
36	02-07-84	28	6.24	0,20	3.33
37	05-07-84	27	6.01	0,19	3.28
38	09-07-84	29	5.99	0,16	2.74
39	16-07-84	28	6.04	0,17	2.94
40	19-07-84	21	6.26	0,19	3.07
41	23-07-84	28	5.75	0,18	3.20
42	26-07-84	20	5.94	0,11	3.90
43	30-01-84	18	5.93	0,17	2.90
44	06-08-84	20	6.16	0,12	1.94
45	13-08-84	27	6.12	0,15	2.51
46	16-08-84	28	6.02	0,13	2.20
48	23-08-84	28	5.46	0,14	2.55
49	27-08-84	28	5.50	0,14	2.64
50	30-08-84	26	6.02	0,16	2.70
51	03-09-84	27	6.10	0,12	1.90
52	06-09-84	26	6.11	0,20	3.42
53	13-09-84	28	6.13	0,18	2.93
54	20-09-84	25	6.08	0,10	1.75

Ministério da Saúde

Fundação Oswaldo Cruz



FIOCRUZ

PROD #	DATE	QUANTITY OF BOTTLES	AVERAGE TITRE	STANDARD DEVIATION	COEFFICIENT OF VARIABILITY
55	24-09-84	27	6.14	0,17	2.73
56	27-09-84	25	6.14	0,20	3.21
57	01-10-84	24	6.21	0,15	2.41
58	04-10-84	25	6.24	0,16	2.53
59	08-10-84	23	6.26	0,19	3.00
60	18-10-84	25	6.29	0,14	1.46
62	25-10-84	22	6.18	0,16	2.58
63	29-10-84	26	6.12	0,18	2.91
64	08-11-84	27	5.81	0,22	3.87
70	03-12-84	27	6.11	0,18	3.84
71	06-12-84	29	6.13	0,13	2.23
68	26-11-84	25	5.25	0,20	3.91
69	29-11-84	17	5.32	0,16	3.10
72	10-12-84	28	5.85	0,13	2.31
73	13-12-84	26	5.83	0,16	2.77
77	21-01-85	26	5.79	0,15	2.62
75	07-12-84	27	5.51	0,16	2.95
76	28-12-84	24	5.65	0,26	4.69
78	28-01-85	26	5.60	0,20	3.60
79	31-01-85	27	5.62	0,19	3.37
TOTAL	1087 bottles	25.2	5.94	0,30	5.05

* TITRES EXPRESSED AS LOG PER ML.

TABLE 2

STANDARD AND STABILIZED YELLOW FEVER VACCINE. TITRES BEFORE AND AFTER LYOPHILIZATION

STANDARD VACCINE				STABILIZED VACCINE			
VACCINE LOT	TITRE BEFORE	TITRE AFTER	LOSS	VACCINE LOT	TITRE BEFORE	TITRE AFTER	LOSS
35	6.18	5.80	0,38	41	5.46.	5.13	0,33
36	6.21	5.86	0,35	44	5.48	5.08	0,40
37	5.95	5.03	0,92	50	5.58	5.24	0,34
38	6.10	5.22	0,88	65	5.97	5.70	0,27
39	5.96	5.21	0,75	68	5.84	5.46	0,38
40	5.65	5.14	0,51	71	5.87	5.51	0,36
43	5.84	5.34	0,50	72	5.35	5.14	0,21
49	6.17	5.55	0,62	74	5.20	4.90	0,30
51	5.63	5.17	0,46	77	5.72	5.49	0,23
52	6.32	5.78	0,04				
54	5.95	5.50	0,45	Mean	5.60	5.29	0,31
55	6.24	5.82	0,42	Standard deviation	0.28	0,27	
56	5.47	5.02	0,45	coefficent of variation	5.00%	5.18%	
57	6.48	6.07	0,41				
60	6,47	6,07	0,37				
61	5.95	5.56	0,39				
62	6.27	5.86	0,41				
Mean	6.04	5.52	0,52				
Standard deviation	0.28	0.36					
Coefficent of Variability	4.73%	6.53%					

* TITRES EXPRESSED AS LOG PER ML.

TABLE 3

TITRES OF 42 LOTS OF STANDARD AND STABILIZED YELLOW FEVER VACCINE

TITRES	VACCINE			
	STANDARD		STABILIZED	
.Log per human dose	NUMBER	%	NUMBER	%
3.00 - 3.20	0	-	0	-
3.21 - 3.40	0	-	3	16.7
3.41 - 3.60	4	16.7	6	33.3
3.61 - 3.80	3	12.5	4	22.2
3.81 - 4.00	5	20.8	5	27.8
> 4.0	12	50.0	0	0
TOTAL	24	100.0	18	100.0

TABLE 4

STANDARD AND STABILIZED YELLOW FEVER VACCINE. RESULTS OF THERMO-STABILITY TESTS AS RECOMMENDED BY WHO.[1]

STANDARD VACCINE				STABILIZED VACCINE			
TITRE (x)				TITRE (x)			
Lot	Before	After[x]	Loss	Lot	Before	After	Loss
35	5.31 (x)	4.17	1.14	41	5.55	5.26	0,29
36	5.40	4.65	0.75	44	5.30	5.07	0,23
37	5.51	3.75	1.74	46	5.21	5.00	0,21
38	5.30	3.69	1.61	48	5.54	5.18	0,36
39	5.59	4.25	1.34	50	5.68	5.30	0,38
40	5.73	4.30	1.43	65	5.63	5.24	0,39
42	5.59	4.50	1.09	68	5.45	4.97	0,48
43	5.82	5.00	0.82	69	5.20	4.86	0,34
45	5.78	3.74	2.04	70	4.92	4.43	0,49
47	5.57	4.07	1.50	71	5.11	4.62	0,49
49	6.04	4.30	1.74	72	5.42	5.02	0,40
51	6.11	3.84	2.27	73	5.09	4.95	0,14
52	5.60	2.70	2.90				
53	5.77	2.51	3.26	Mean	5.34	4.99	0,35
54	5.88	2.86	3.02	SD	0.24	0.26	-
55	6.00	4.02	1.98	CV	4.48%	5.21%	-
56	5.67	3.00	2.67				
57	5.63	3.54	2.09				
58	5.78	3.24	2.54				
60	5.75	3.17	2.58				
61	5.84	3.87	1.97				
62	5.98	3.67	2.31				
67	5.12	3.57	1.57				
Mean	5.68	3.75	1.92				
SD	0.25	0.63	-				
CV	4.38%	16.9%	-				

SD= Standard deviation; CV= Coefficient of variation
(1) TRS 594, 1976
(x) Titre expressed as log/ml

TABLE 5

PHYSICAL, CHEMICAL AND BIOLOGICAL CHARACTERISTICS OF STABILIZED YELLOW FEVER VACCINE

VACCINE LOT	POTENCY	% RESIDUAL HUMIDITY	PROTEIN NITROGEN	AVERAGE WEIGHT	STERILITY	INNOCUITY	IDENTITY	THERMO-STABILITY
41	3.44	2.00	0,14	80.77	ok	ok	ok	0,29
44	3.38	1.81	0,14	77.40	ok	ok	ok	0,23
46	3.69	1.48	0,17	84.65	ok	ok	ok	0,21
48	3.52	1.69	0,21	75.67	ok	ok	ok	0,36
50	3.44	1.48	0,22	73.75	ok	ok	ok	0,38
65	4.0	1.68	0,15	76.70	ok	ok	ok	0,39
68	3.76	1.65	0,15	70.83	ok	ok	ok	0,48
69	3.43	2.29	0,13	79.71	ok	ok	ok	0,34
70	3.25	2.39	0,14	72.90	ok	ok	ok	0,49
71	3.85	2.46	0,14	79.05	ok	ok	ok	0,49
72	3.44	2.17	0,13	74.21	ok	ok	ok	0,40
73	3.72	2.06	0,16	75.09	ok	ok	ok	0,24

Potency expressed as log per human dose.

Protein nitrogen expressed as miligrames per human dose.

Thermostability expressed as loss in titer after exposure during two weeks at 37ºC.

TABLE 6

STABILITY OF RECONSTITUTED YELLOW FEVER VACCINE INCUBATED IN ICE BATH AND 37ºC

DILUENT / INCU-BATION	PBS		PBS PLUS GELATINE		PBS PLUS GELISATE		DISTILLED WATER	
	37º C	ICE BATH	37ºC	ICE BATH	37ºC	ICE BATH	37ºC	ICE BATH
Initial	4.00*	4.00	4.36	4.36	4.10	4.10	4.12	4.12
3 hours	- **	4.02	2.00	4.00	1.50	4.16	3.65	4.24
6 hours	-	4.25	-	4.27	-	4.50	3.81	4.33

* Titres expressed as log per human dose

** No plaques in 10^{-1} dilution

TABLE 7

STANDARD YELLOW FEVER VACCINE. STABILITY OF RECONSTITUTED VACCINE AT 37ºC WITH TWO DILUENTS

DILUENT / INCUBATE AT 37ºC	DISTILLED WATER				P B S			
	Pure	WITH GELISATE 2%	WITH GELATIN 2%	WITH SERUM 2%	Pure	WITH GELISATE 2%	WITH GELATIN 2%	WITH SERUM 2%
Initial	3.89*	3.82	3.79	3.77	3.93	3.76	3.60	3.91
1 hour	3.69	3.71	3.02	3.70	2.70	2.09	2.94	4.12
3 hours	3.91	3.24	2.17	3.76	-	1.70	2.90	3.67

* Titres expressed as log per human dose

** No plaques at 10^{-1} dilution

TABLE 8

STANDARD AND STABILIZED YELLOW FEVER VACCINES. STABILITY OF RECONSTITUTED VACCINE IN DISTILLED WATER INCUBATED IN 37ºC AND ICE BATH

VACCINE	BEGINNING	3 hours in ice bath	3 hours at 37ºC
01	3.63 (x)	4.08	3.55
36	4.00	3.97	3.40
44	3.54	3.51	3.25
65	3.90	3.86	327

(x) Titers expressed as log per human dose

Vaccines 01 and 36 are standard; vaccines 44 and 65 are stabilized.

TABLE 9

YELLOW FEVER VACCINE. LOSS OR GAIN IN TITRE OF STABILIZED AND STANDARD VACCINES RECONSTITUTED WITH VARIOUS DILUENTS AFTER INCUBATION AT 37ºC DURING 3 HOURS

DILUENT	STANDARD VACCINE	STABILIZED VACCINE
Distilled water	+ 0,40	- 0,04
0,15 M Saline	- 1,29	- 0,86
0,03 M Saline	- 0,30	- 0,33
0,001M Sucrose	- 1,40	- 0,49
0,01 M Glutamate	- 1,27	- 1,03
0,01 M Arginine	- 1,62	- 1,49
Sucrose plus Glutamate	- 1,27	- 1,34
Sucrose plus Arginine	- 1,37	- 1,47
Glutamate plus Arginine	- 1,58	- 1,03

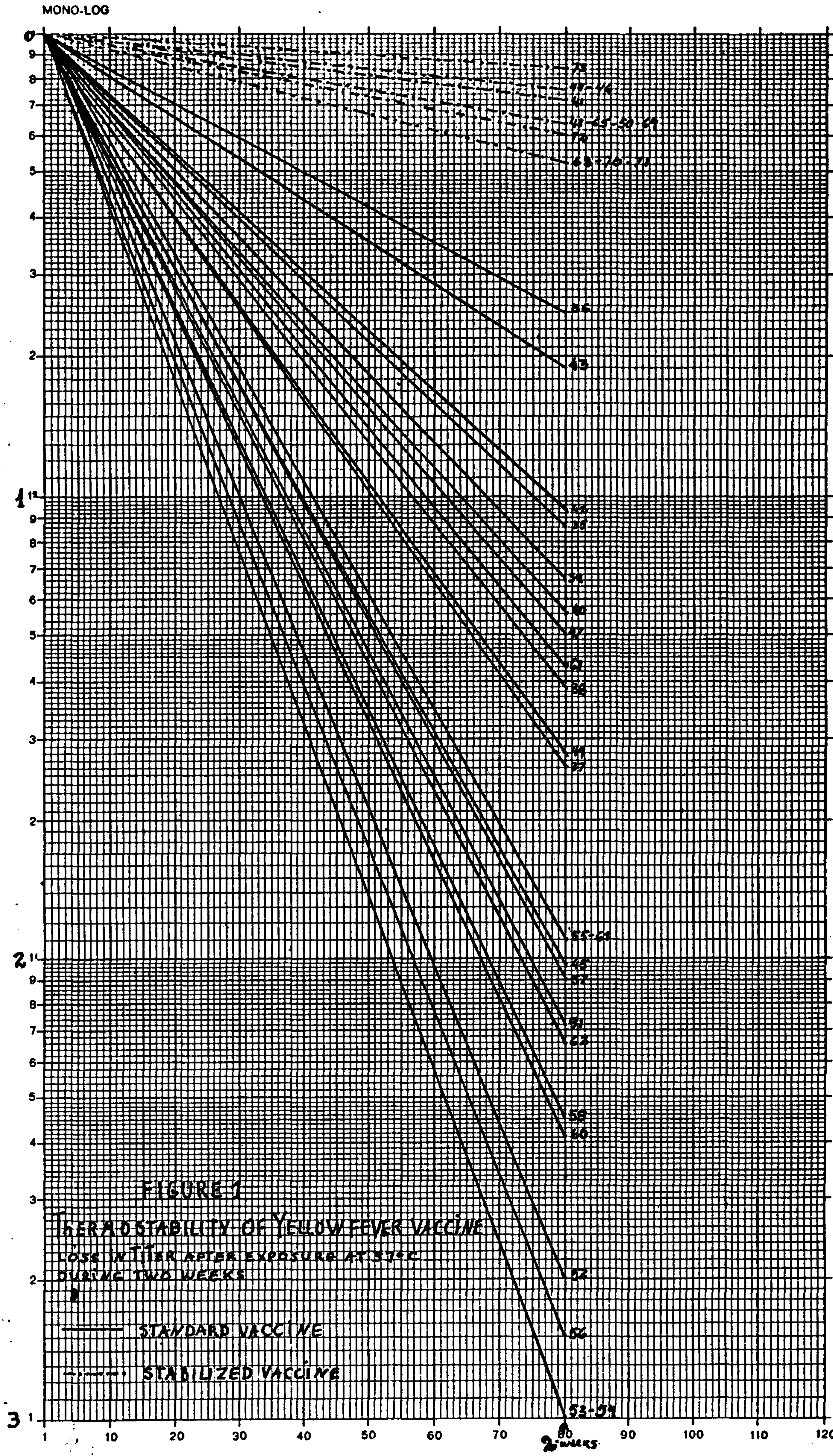


FIGURE 1

THERMOSTABILITY OF YELLOW FEVER VACCINE

LOSS IN TITER AFTER EXPOSURE AT 37°C DURING TWO WEEKS

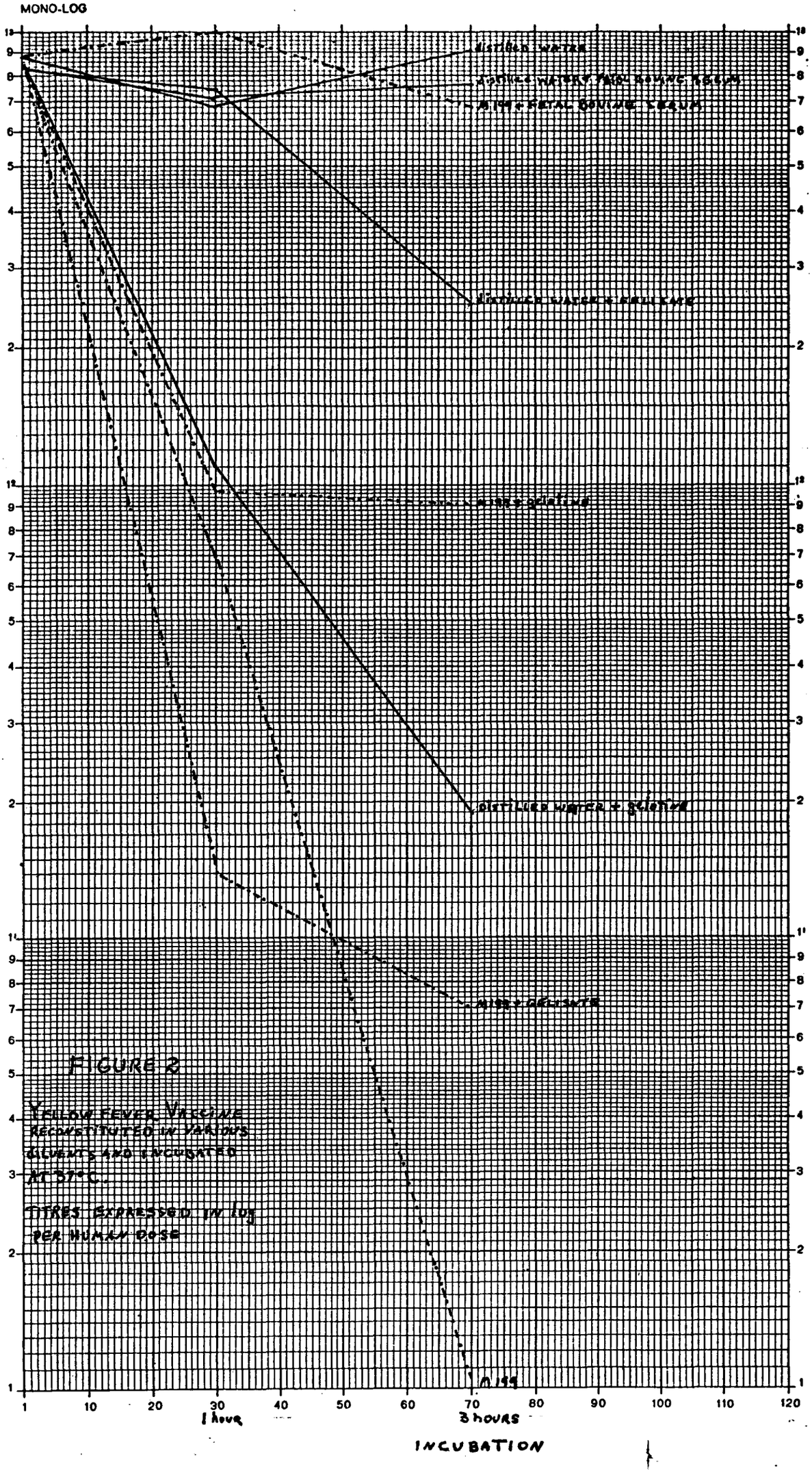


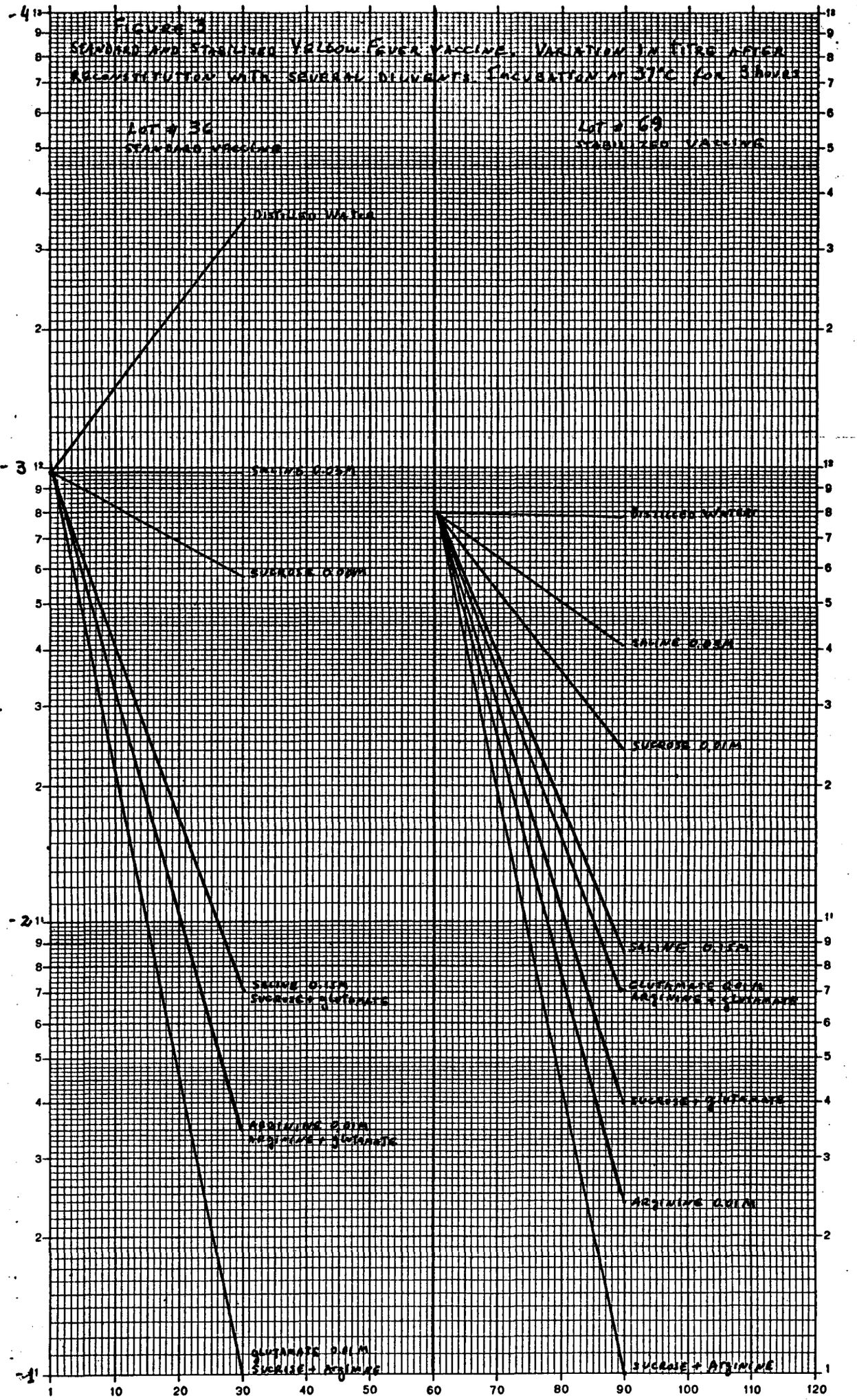
FIGURE 2

YELLOW FEVER VACCINE RECONSTITUTED IN VARIOUS DILUENTS AND INCUBATED AT 37°C.

TITRES EXPRESSED IN $\log_{10}$ PER HUMAN DOSE

MONO-LOG

FIGURE 3
SUCROSE AND STABILISED YELLOW FEVER VACCINE. VARIATION IN TITRE AFTER RECONSTITUTION WITH SEVERAL DILUENTS. INCUBATION AT 37°C FOR 5 HOURS



FINANCIAL STATEMENTS

CONVÊNIO CENTRO INTERNACIONAL DE PESQUISAS

PARA O DESENVOLVIMENTO - CANADÁ

PROJETO: FEBRE AMARELA (AMÉRICA LATINA)

POSIÇÃO EM: 12.06.85

DATA	RECEITA		DESPESA			TOTAL
			MATERIAL DE CONSUMO	A PAGAR	PAGO	
29.09.83 -	Importância recebida conf. Aviso de Crédito 3916066 de 22.09.83 e depositado na c/c 71.556-5 do Banco do Brasil S/A	927.535	EMPENHADOS NO ANO DE 1984			
			Material Gráfico	-	744.240	-
			Material de Escritório	-	112.101	856.341
14.02.85 -	Importância recebida conf. Aviso de Crédito 3916540 de 11.02.85 e depositado na c/c 71.556-5 do Banco do Brasil S/A	4.470.550	EMPENHADOS NO ANO DE 1985			
			Materiais Diversos	4.541.744	-	4.541.744
TOTAL		5.398.085	TOTAL	-	-	5.398.085

Rio de Janeiro, 12 de junho de 1985

JOSÉ CARLOS SANTIAGO
Gerente Serviço de Contabilidade
Contador CRC - 020.108-6

Dr. AKIRA HOMMA
Superintendente de Bio-Manguinhos

MISC/ajmr.

B U D G E T

FIOCRUZ ADMINISTERED FUNDS (IN CRUZEIROS)
FUNDS RECEIVED FROM IDRC IN CANADIAN DOLLARS

D A T E	F U N D S	EXPENSES			TOTAL
		CONSUMABLE SUPPLIES	TO BE PAID	PAID	
28.09.83	Received from IDRC by credit bill 3916066 and deposited at Banco do Brasil account in 71.556-5 ..... 927.535	1984			
		Graphic materials	-	744.240	-
		Office materials	-	112.101	856.341
14.02.85	Received from IDRC by credit bill 3916540 and deposited at Banco do Brasil account in 71.556-5 ..... 4.470.550	1985			
		Several office materials	4.541.744	-	4.541.744
T O T A L	5.398.085	T O T A L	-	-	5.398.085

Rio de Janeiro, 12 de junho de 1985.