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APPENDICES

Appendix I

Raising and Maintianing of Mice

Mice are usually maintained in boxed with pine shapings,which are cleaned twice a week. Recommended cage sizes are given below;

Desired area per animal (square foot)	Unit	Unit size WXDXH (inches)
-	Cage for 2	5 X 9 X 5
0.05-0.10	Cage for 5-10	8X12 X 5
0.10-0.70	Cage for 10-20	12X16X 5

Table 30 Space suggested for the routine housing of common laboratory animals.

Pelleted chow (free of antibiotics) should be provided in an overhead wire mesh popper, commonly built into the top of the cage. Water bottles are atteched,with airtight rubber closures fitted with stainless steel or glass tubing to serve as nipples. Although elaborate water supply systems can be installed with tongue-activates valves at each cage "station",individual water bottles offer flexibility in using various additives,such as antibiotics, during experimental work. Instead of plain drinking water acidulated water has been introduced to reduce bacterial growth and cross contamination in the drinking water. This type of fluid intake has been used in breeding many successive generations of mice, and it is to be recommended: 3 ml of N.HCl is added per liter of water.

Appendix II

Bleeding Mothod

Cadiac Puncture Sacrific in Mice

To obtain the maximun volume of blood,chloroform is preferred for anesthesia,since it cause dilation, first of the left atrium and later of the entire heart. The mouse is put into a beaker with a wire-grid bottum under which a pledget of chloroform-moistened cotton is present, the top closed by petridish. At a deep level of anesthesia, the animal is removed and is pinned by the feet,ventral side up and taut, onto a piece of heavy pressed cork. The chest area is washed with 70% alcohol. Blood may be obtained from the heart by either of the following two methods,using aseptic technique.

1) Withdrawal From Closed Chest by Syringe and Needle

The skin is deflected from the chest wall, and a 27 gaug (short-bovel) needle attached to a 2 ml. syringe is inserted between the left intercostal spaces at an angle designed to penetrate the left ventricle of the heart. When blood appears in the barrel of the syringe ,the piston should be withdrawn very slowly, since too great negative pressure tends to collapse the heart wall. With experience one may eventually expect to obtain in this manner approximately 1.5 to 2 ml of blood from a 20 to 25 g mouse. An inexperienced operator should secure at least 0.75 ml of blood. The operator must be able to extract the blood expeditiously to avoid clotting, and care must be taken in pressing the blood into the receiving vessel to avoid hemolysis.

2) Removal from Open Chest by Capillary Pipette.

This method is technically easier,although it does not allow axact measurement of blood volume. Commercial disposible Pastuer pipettes (6 inches long) are plugged with cotton and dry sterilized. Immediately before use the capillary tip is flamed in a Bunsen burner and pulled out finely so that the wall is rather rigid and the bore approximates a 20 to 22 guage needle. It is broken off, preferably leaving a jagged end more easily able to penetrate the heart wall. A series of pipettes may be laid out on a rack in an area adjacent to a Bunsen burner where the conditions can be considered aseptic. Just before use,each pipette is fitted woth a rubber teat. The mouse is anesthetized with chloroform and prepared as in section 1) just cited, and the chest wall is opened. While the heart still beats ,the pipette is introduced into the wall of the left ventricle,and blood is withdrawn slowly. As with the closed-chest method practice is essential to obtain the maximum amount of blood (1.5 to 2 ml) without clotting or hemolysis.

Appendix III

Preparation of Serum from Blood

In most immunological procedure serum is employed in preference to plasma. Serum is obtained by allowing blood to clot,freely the clot from the walls of the container, allowing the clot to retract,collecting the expressed serum and removing any loose blood cells by centrifugation

Clotting and retraction take place best at 37° C. For this purpose immersion of the container in a water bath is very much more efficient than the use of an incubator. To obtain the maximum yield of serum, clots adherent to the container wall should be freed as soon as they are firm enough. Contraction for 2 hours in a water bath is sufficient for most of the serum to be expressed. Only a small additional yield is obtained further incubation either in the water bath or by overnight storage in the cold.

Appendix IV

Statistical Analysis

a) Pyrogen test

1) Result from table 9 and 10 were filled in contingency table as table 31, 32 ,and 33.

Table 31 Contingency table of survived mice in first month.

Days	TTMA	TTMB	TTMC	TT+TTMA	TT+TTMB	TT+TTMC	TT	$\Sigma x_{.j}$	$\Sigma x_{.j}^2$	$x_{.j}$
0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0
7	0	0	0	2	3	2	3	10	100	1.43
15	0	2	3	6	6	5	5	28	784	4
30	3	7	9	10	10	10	9	58	3364	8.29
Σx_i	3	9	12	18	19	17	17	96		
Σx_i^2	9	81	144	324	361	289	289			
x_i	0.6	1.8	2.4	3.6	3.8	3.4	3.4			

Table 32 Contingency table of survived mice during days 45 and 90.

Days	TTMA	TTMB	TTMC	TT+TTMA	TT+TTMB	TT+TTMC	TT	Σx_j	Σx_j^2	x_j
45	5	8	10	10	10	10	10	63	3969	9.00
60	8	10	10	10	10	10	10	68	4624	9.71
75	10	10	10	10	10	10	7	67	4489	9.57
90	10	9	7	10	10	3	3	52	2704	7.43
Σx_i	33	37	37	40	40	33	30	250		
Σx_i^2	1089	1369	1369	1600	1600	1089	900			
x_i	8.25	9.25	9.25	10.00	10.00	8.25	7.5			

Table 33 Contingency table of survived mice during days 105 and 180.

Days	TTMA	TTMB	TTMC	TT+TTMA	TT+TTMB	TT+TTMC	TT	$\Sigma x_{.j}$	$\Sigma x_{.j}^2$	$x_{.j}$
105	10	10	3	10	10	3	0	46	2116	6.57
120	10	10	1	10	10	1	0	42	1764	6.00
140	8	10	0	8	9	0	0	35	1225	5.00
160	7	10	0	7	10	0	0	34	1156	4.86
180	5	10	0	4	10	0	0	29	841	4.14
Σx_{i}	40	50	4	39	49	4	0	186		
Σx_{i}^2	1600	2500	16	1521	2401	16	0			
x_{i}	8	10	0.8	7.8	9.8	0.8	0			

<u>Hypothesis</u>

Treatment.

H_{A0} = There are not significantly difference in no. of survived mice among tetanus toxoid preparation.

H_{Aa} = There are significantly difference in no. of survived mice among tetanus toxoid preparation.

Block.

H_{B0} = There are not significantly difference in no. of survived mice in each period of time.

H_{Ba} = There are significantly difference in no. of survived mice in each period of time.

Calculations data from table 32;

Σx^2_{ij} = 645

CT. (Correction Term) = $\frac{(\Sigma x_i)^2}{N} = \frac{(96)^2}{35} = 263.31$

SS_{total} (Total sum of squares) $= \Sigma x_{ij}^2 - CT$

$= 645 - 263.31$

$= 381.69$

SS_{block} (Block sum of squares) $= \frac{\Sigma(\Sigma x_j^2)}{n_j} - CT$

$= \frac{(0+0+100+784+3364)}{7} - 263.31$

$= 343.55$

$SS_{treatment}$ (Treatment sum of squares) $= \frac{\Sigma(\Sigma X_i^2)}{n_i} - CT$

$= \frac{(9+81+144+324+361+289+289)}{5} - 263.31$

$= 36.09$

Table 34 ANOVA table of survived mice during days 0 and 30.

Source	df	SS	MS	F
Treatment	6	36.09	6.015	70.43
Block	4	343.55	85.8875	1005.71
Residual	24	2.05	0.0854	
Total	34	381.69		

Treament; from table 43 , $F_{0.05}(6,24) = 2.49$

$70.43 > F_{0.05}$; Reject the null hypothesis ($H_{1\ o}$)

Block; from table 43 , $F_{0.05}(4,24) = 2.76$

$1005.71 > F_{0.05}$; Reject the null hypothesis ($H_{1\ o}$)

Hence, there are significantly difference in no. of survived mice both among tetanus toxoid preparations and periods of time.

Duncan's New Multiple Range Test for treatment (Data from table 31)

$S_x = \sqrt{0.0854/5} = 0.1307$

df of error = 24

P value	2	3	4	5	6	7
SSR	2.92	3.07	3.15	3.22	3.28	3.31
$LSR = SSR X S_x$	0.3816	0.4012	0.4117	0.4208	0.4287	0.4326

Preparation	TTMA	TTMB	TTMC	TT+TTMC	TT	TT+TTMA	TT+TTMB
X	0.6	1.8	2.4	3.4	3.4	3.6	3.8

TT+TTMB,TTMA	3.8-0.6 = 3.2 > 0.4326	S
TT+TTMB,TTMB	3.8-1.8 = 2.0 > 0.4287	S
TT+TTMB,TTMC	3.8-2.4 = 1.4 > 0.4208	S
TT+TTMB,TT+TTMC	3.8-3.4 = 0.4 < 0.4117	NS
TT+TTMB,TT	3.8-3.4 = 0.4 < 0.4012	NS
TT+TTMB,TT+TTMA	3.8-3.6 = 0.2 < 0.3816	NS

TT+TTMA,TTMA	3.6-0.6 = 3.0 > 0.4287	S
TT+TTMA,TTMB	3.6-1.8 = 1.8 > 0.4208	S

TT+TTMA,TTMC	3.6-2.4 = 1.2 > 0.4117	S
TT+TTMA,TT+TTMC	3.6-3.4 = 0.2 > 0.4012	NS
TT+TTMA,TT	3.6-3.4 = 0.2 > 0.3816	NS
TT,TTMA	3.4-0.6 = 2.8 > 0.4208	S
TT,TTMB	3.4-1.8 = 1.6 > 0.4117	S
TT,TTMC	3.4-2.4 = 1.0 > 0.4012	S
TT,TT+TTMC	3.4-3.4 = 0.0 < 0.3816	NS
TT+TTMC,TTMA	3.4-0.6 = 2.8 > 0.4117	S
TT+TTMC,TTMB	3.4-1.8 = 1.6 > 0.4012	S
TT+TTMC,TTMC	3.4-2.4 = 1.0 > 0.3816	S
TTMC,TTMA	2.4-0.6 = 1.8 > 0.4012	S
TTMC,TTMB	2.4-1.8 = 0.6 > 0.3816	S
TTMB,TTMA	1.8-0.6 = 1.2 > 0.3816	S

Duncan's New Multiple Range Test for block (Data from table 31)

$S_{\bar{x}} = \sqrt{0.0854/7} = 0.11045$

df of error = 24

P value	2	3	4	5
SSR	2.92	3.07	3.15	3.22
LSR=SSR×$S_{\bar{x}}$	0.3225	0.3391	0.3479	0.3556

DAYS	0	3	7	15	30
X	0	0	1.43	4	8.29

Days 30,0 = 8.29-0 = 8.29 > 0.3556 S

30,3 = 8.29-0 = 8.29 > 0.3479 S

30,7 = 8.29-1.43 = 6.86 > 0.3391 S

30,15 = 8.29-4.0 = 4.29 > 0.3225 S

15,0 = 4.0-0 = 4.0 > 0.3479 S

15,3 = 4.0-0 = 4.0 > 0.3391 S

15,7 = 4.0-1.43 = 2.57 > 0.3225 S

7,0 = 1.43-0 = 1.43 > 0.3391 S

7,3 = 1.43-0 = 1.43 > 0.3391 S

3,0 = 0-0 = 0 < 0.3225 NS

Data from table 32

$\Sigma x^{2}_{ij} = 2350$

$CT = \frac{(\Sigma X_{i}^{2})}{N} - CT = \frac{(2350)^{2}}{28} = 2232.14$

$SS_{total} = \Sigma X_{ij}^{2} - CT = 2350-2232 = 118$

$SS_{block} = \frac{(\Sigma X_{i}^{2})}{n_{i}} - CT = \frac{15786}{7} - 2232 = 23.14$

$SS_{treatment} = \frac{\Sigma(\Sigma X^{2}_{j})}{n_{j}} - CT = \frac{9016}{4} - 2232 = 22$

Table 35 ANOVA table of survived mice during days 45 and 90.

Source	df	SS	MS	F
Treatment	6	22.00	3.6667	0.9059
Block	3	23.14	7.7133	1.9056
Residual	18	72.86	4.0478	
Total	27	118		

Treament; from table 24 , $F_{0.05}(6,27) = 3.87$

$0.9059 < F_{0.05}$; Accept the null hypothesis ($H_{1\ 0}$)

Block; from table 24 , $F_{0.05}\ (3,27) = 3.10$

$1.90561 < F_{0.05}$; Accept the null hypothesis ($H_{1\ a}$)

Hence, there are not significantly difference in no. of survived mice both among tetanus toxoid preparations and periods of time.

<u>Data from table 33</u>

$$\Sigma X^2_{ij} = 1668$$

$$CT = \frac{(\Sigma X_i^2)}{N} - CT = \frac{(186)^2}{35} = 988.46$$

$$SS_{total} = \Sigma X_{ij}^2 - CT = 1668-988.46 = 679.54$$

$$SS_{block} = \frac{(\Sigma X_i^2)}{n_i} - CT = \frac{7102}{7} - 988.46 = 26.11$$

$$SS_{treatment} = \frac{\Sigma(\Sigma X^2_{.j})}{n_j} - CT = \frac{8054}{5} - 988.46 = 622.34$$

Table 36 ANOVA table of survived mice during days 105 and 180.

Source	df	SS	MS	F
Treatment	6	622.34	103.7233	80.0705
Block	4	26.11	6.5275	5.0389
Residual	24	31.09	1.2954	
Total	34	679.54		

Treament; from table 43 , $F_{0.05}(6,24) = 2.49$

80.8908> $F_{0.05}$; Reject the null hypothesis ($H_{1\ o}$)

Block; from table 43 , $F_{0.05}$ (4,24) = 2.75

5.0389 > $F_{0.05}$; Reject the null hypothesis ($H_{1\ a}$)

Hence, there are significantly difference in no. of survived mice both among tetanus toxoid preparations and periods of time.

Duncan's New Multiple Range Test for treatment (Data from table 33)

$S_x = \sqrt{1.2954/5} = 0.50899$

df of error = 2

P value	2	3	4	5	6	7
SSR	2.92	3.07	3.15	3.22	3.28	3.31
LSR=SSRXS_x	1.4863	1.5626	1.6033	1.6389	1.6695	1.6848

Preparation	TT	TT+TTMC	TTMC	TT+TTMA	TTMA	TT+TTMB	TTMB
X	0	0.8	0.8	7.8	8.0	9.8	10.0

TTMB,TT = 10.0-0 = 10 > 1.6848 S

TTMB,TT+TTMC = 10.0-0.8 = 9.2 > 1.6695 S

TTMB,TTMC = 10.0-0.8 = 9.2 > 1.6389 S

TTMB,TT+TTMA = 10.0-7.8 = 2.2 > 1.6033 S

TTMB,TTMA = 10.0-8.0 = 2.0 > 1.5626 S

TTMB,TT+TTMB = 10.0-9.8 = 0.2 < 1.4863 NS

TT+TTMB,TT = 9.8-0.0 = 9.8 > 1.6695 S

TT+TTMB,TT+TTMC = 9.8-0.8 = 9.0 > 1.6389 S

TT+TTMB,TTMC	=	9.8-0.8 = 9.0 > 1.6033	S
TT+TTMB,TT+TTMA	=	9.8-7.8 = 2.0 > 1.5626	S
TT+TTMB,TTMA	=	9.8-8.0 = 1.8 > 1.4863	S
TTMA,TT	=	8.0-0.0 = 8.0 > 1.6389	S
TTMA,TT+TTMC	=	8.0-0.8 = 7.2 > 1.6033	S
TTMA,TTMC	=	8.0-0.8 = 7.2 > 1.5626	S
TTMA,TT+TTMA	=	8.0-7.8 = 0.2 < 1.4863	NS
TT+TTMA,TT	=	7.8-0.0 = 7.8 > 1.6033	S
TT+TTMA,TT+TTMC	=	7.8-0.8 = 7.0 > 1.5626	S
TT+TTMA,TTMC	=	7.8-0.8 = 7.0 > 1.4863	S
TTMC,TT	=	0.8-0.0 = 0.8 < 1.5626	NS
TTMC,TT+TTMC	=	0.8-0.8 = 0.0 < 1.4863	NS
TT+TTMC,TT	=	0.8-0.0 = 0.8 < 1.4863	NS

*** During days 105 and 180 most preparations difference in no. of survived mice except **TT and TT+TTMB**

TTMA and TT+TTMA

TT and TTMC , TT+TTMC

TTMC and TT+TTMC.

Duncan's New Multiple Range Test for block (Data from table 33)

$S_{\bar{x}} = \sqrt{1.2954/7} = 0.4302$

df of error = 24

P value	2	3	4	5
SSR	2.92	3.07	3.15	3.22
$LSR = SSR \times S_{\bar{x}}$	1.2561	1.3207	1.3551	1.3852

DAYS	180	160	140	120	105
$\bar{X}$	4.14	4.86	5.00	6.00	6.57

Days 105,180 = 6.57-4.14 = 2.43 > 1.3852 S

105,160 = 6.57-4.86 = 1.71 > 1.3551 S

105,140 = 6.57-5.00 = 1.57 > 1.3207 S

105,120 = 6.57-6.00 = 0.57 > 1.2561 S

120,180 = 6.00-4.14 = 1.86 > 1.3551 S

120,160 = 6.00-4.86 = 1.14 > 1.3207 S

120,140 = 6.00-5.00 = 1.00 < 1.2561 NS

140,180 = 5.00-4.14 = 0.86 < 1.3207 NS

140,160 = 5.00-4.86 = 0.14 < 1.2561 NS

160,180 = 4.86-4.14 = 0.72 < 1.2561 NS

Antibody determination

Results from table 19,20 were filled in contingency table as table 37 ,38 ,and 39.

Table 37 Contingency table of mice's titers in first month.

Days	TTMA	TTMB	TTMC	TT+TTMA	TT+TTMB	TT+TTMC	TT	Σx_{j}	Σx_{j}^{2}	x_{j}
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.07	0.15	0.12	0.15	0.13	0.62	0.384	0.09
7	0.00	0.25	0.45	0.80	0.85	0.80	0.63	3.78	14.288	0.54
15	0.06	0.65	0.75	1.60	1.60	1.60	1.00	7.26	52.708	1.04
30	0.10	1.80	2.00	2.60	2.60	2.80	1.40	13.3	176.89	1.90
Σx_{i}	0.16	2.70	3.27	5.15	5.17	5.35	3.16	24.96		
Σx_{i}^{2}	0.03	7.29	10.69	26.54	26.73	28.62	9.99			
x_{i}	0.03	0.54	0.65	1.03	1.03	1.07	0.63			

Table 38 Contingency table of mice' s titers during days 45 and 90.

Days	TTMA	TTMB	TTMC	TT+TTMA	TT+TTMB	TT+TTMC	TT	$\Sigma x_{.j}$	$\Sigma x_{.j}^{2}$	$x_{.j}$
45	0.45	2.60	2.80	3.20	3.40	3.40	2.10	17.95	322.20	2.56
60	0.75	3.20	3.40	3.20	3.40	2.50	2.00	18.45	340.40	2.64
75	1.80	3.40	2.00	3.20	2.80	1.50	2.00	16.70	278.85	2.39
90	2.00	3.40	1.00	2.20	2.50	0.85	0.55	12.50	156.25	1.79
Σx_{i}	5.00	12.60	9.20	11.80	12.10	8.25	6.65	65.60		
Σx_{i}^{2}	25.00	158.76	84.64	139.24	146.41	85.56	44.22			
x_{i}	1.25	3.15	2.30	2.95	3.02	2.06	1.66			

Table 39 Contingency table of mice's titers during days 105 and 180.

Days	TTMA	TTMB	TTMC	TT+TTMA	TT+TTMB	TT+TTMC	TT	$\Sigma x_{.j}$	$\Sigma x_{.j}^2$	$x_{.j}$
105	1.70	2.40	1.80	1.50	2.20	1.50	0.45	11.55	133.4	1.65
120	1.20	2.10	1.10	0.85	1.90	0.85	0.06	8.06	64.96	1.15
140	0.95	1.85	0.80	0.50	1.40	0.05	0.00	6.00	36.00	0.86
160	0.85	1.50	0.45	0.40	1.10	0.40	0.00	4.70	22.09	0.67
180	0.78	1.20	0.40	0.43	1.50	0.43	0.00	4.74	22.47	0.68
$\Sigma x_{i.}$	5.48	9.05	4.55	3.68	8.18	3.68	0.51	35.59		
$\Sigma x_{i.}^2$	30.03	81.90	20.70	13.54	65.61	13.54	0.260			
$x_{i.}$	1.09	1.81	0.91	0.73	1.62	0.73	0.102			

Hypothesis

Treatment.

$H_{A\,o}$ = There are not significantly difference in titers level among tetanus toxoid preparations.

$H_{A\,a}$ = There are significantly difference in titers level among tetanus toxoid preparation.

Block.

$H_{B\,o}$ = There are not significantly difference in titers level in each period of time.

$H_{B\,a}$ = There are significantly difference in titers level in each period of time.

<u>Calculations</u> data from table 37 ;

$\Sigma x^2_{ij} = (24.96)^2$

CT. (Correction Term) = $\frac{(\Sigma x_{i})^2}{N} = \frac{(24.96)^2}{35} = 17.800$

SS_{total} (Total sum of squares) $= \Sigma x_{ij}^2 - CT$

$= 42.9842 - 17.800$

$= 25.1842$

SS_{block} (Block sum of squares) $= \frac{\Sigma(\Sigma x_{j}^2)}{n_j} - CT$

$= \frac{(0+0.3833+14.2884+52.7076+176.8900)}{7} - 17.800$

$= \frac{244.2704}{7} - 17.8 = 17.0958$

$SS_{treatment}$ (Treatment sum of squares) $= \frac{\Sigma(\Sigma X_{i}^2)}{n_i} - CT$

$= \frac{(0.0260+2.29+10.69+26.52+26.73+28.62=9.9856)}{5} - CT$

$= \frac{110.068}{5} - 17.80 = 4.2136$

Table 40 ANOVA table of mice's titers in first month.

Source	df	SS	MS	F
Treatment	6	4.2136	0.7023	4.3486
Block	4	17.0957	4.2739	26.4638
Residual	24	3.8749	0.1615	
Total	34	25.1842		

Treament; from table 24 , $F_{0.05}(6,24) = 2.49$

$4.3486 > F_{0.05}$; Reject the null hypothesis $(H_{1\ 0})$

Block; from table 24 , $F_{0.05}(4,24) = 2.76$

$26.46381 > F_{0.05}$; Reject the null hypothesis $(H_{1\ a})$

Hence, there are significantly difference of titers level both among tetanus toxoid preparations and periods of time.

Duncan's New Multiple Range Test for treatment (Data from table 37)

$S_x = \sqrt{0.1615/5} = 0.1797$

df of error = 24

P value	2	3	4	5	6	7
SSR	2.92	3.07	3.15	3.22	3.28	3.31
$LSR=SSR*S_x$	0.5247	0.5517	0.5661	0.5786	0.5894	0.5948

Preparation	TTMA	TTMB	TT	TTMC	TT+TTMA	TT+TTMB	TT+TTMC
X	0.03	0.54	0.63	0.65	1.030	1.034	1.07

TT+TTMC,TTMA	1.07-0.032	=	1.038	>	0.5948	S
TT+TTMC,TTMB	1.07-0.54	=	0.53	<	0.5894	NS
TT+TTMC,TT	1.07-0.632	=	0.438	<	0.5786	NS
TT+TTMC,TTMC	1.07-0.654	=	0.416	<	0.5661	NS
TT+TTMC,TT+TTMA	1.07-1.03	=	0.040	<	0.5517	NS
TT+TTMC,TT+TTMB	1.07-1.034	=	0.036	<	0.5247	NS

TT+TTMB,TTMA	1.034-0.032	=	1.002	>	0.5894	S
TT+TTMB,TTMB	1.034-0.54	=	0.494	<	0.5786	NS
TT+TTMB,TT	1.034-0.632	=	0.402	<	0.5661	NS
TT+TTMB,TTMC	1.034-0.654	=	0.380	<	0.5517	NS
TT+TTMB,TT+TTMA	1.034-1.030	=	0.004	<	0.5247	NS
TT+TTMA,TTMA	1.03-0.032	=	0.998	>	0.5786	S
TT+TTMA,TTMB	1.03-0.540	=	0.490	<	0.5661	NS
TT+TTMA,TT	1.03-0.632	=	0.398	<	0.5117	NS
TT+TTMA,TTMC	1.03-0.654	=	0.376	<	0.5247	NS
TTMC,TTMA	0.654-0.032	=	0.622	>	0.5661	S
TTMC,TTMB	0.654-0.541	=	0.114	<	0.5517	NS
TTMC,TT	0.654-0.632	=	0.022	<	0.5247	NS
TT,TTMA	0.632-0.032	=	0.600	>	0.5517	S
TT,TTMB	0.632-0.540	=	0.092	<	0.5247	NS
TTMB,TTMA	0.540-0.032	=	0.508	<	0.5247	NS

Duncan's New Multiple Range Test for block (Data from table 37)

$S_x = \sqrt{0.1615/7} = 0.1519$

df. of error = 24

P value	2	3	4	5
SSR	2.92	3.07	3.15	3.22
$LSR = SSR \times S_x$	0.4435	0.4663	0.4785	0.4891

DAYS	0	3	7	15	30
X	0	0.089	0.540	1.037	1.900

Days 30,0 = 1.90 - 0 = 1.90 > 0.4891 S

30,3 = 1.90 - 0.089 = 1.811 > 0.4785 S

30,7 = 1.90 - 0.540 = 1.360 > 0.4663 S

30,15 = 1.90 - 1.037 = 0.863 > 0.4435 S

15,0 = 1.037 -0 = 1.037 > 0.4785 S

15,3 = 1.037 - 0.089 = 0.948 > 0.4663 S

15,7 = 1.037 - 0.540 = 0.497 > 0.4435 S

7,0 = 0.54 - 0 = 0.54 > 0.4435 S

7,3 = 0.54 - 0.089 = 0.451 > 0.4435 S

3,0 = 0.089 -0 = 0.089 < 0.3225 NS

Data from table 38

$\Sigma X^2_{ij} = (65.6)^2$

$CT = \frac{(\Sigma X_i^2)}{N} - CT = \frac{(65.6)^2}{28} = 153.69$

$SS_{total} = \Sigma X_{ij}^2 - CT = [(0.45)^2+(2.6)^2+(2.8)^2+---+(2.5)^2+(0.85)^2]-CT$

$= 178.79-153.69 = 25.1$

$SS_{block} = \frac{(\Sigma X_i^2)}{n_i} - CT = \frac{332.2+340.40+278.85+156.25}{7} - 153.69 = 4.554$

$SS_{treatment} = \frac{\Sigma(\Sigma X^2_j)}{n_j} - CT$

$= \frac{25+158.76+84.64+139.24+146.41+85.56+44.22}{4} - 153.69$

$= 170.96-153.69 = 17.27$

Table 41 ANOVA table of mice titer's during days 45 and 90.

Source	df	SS	MS	F
Treatment	6	17.27	2.8783	15.7975
Block	3	4.55	1.5167	8.3244
Residual	18	3.28	0.1822	
Total	27	25.1		

Treament; from table 43 , $F_{0.05}(6,18) = 2.60$

$15.7975 > F_{0.05}$;Reject the null hypothesis $(H_{1\ 0})$

Block; from table 43 , $F_{0.05}(3,18) = 3.10$

$8.32441 < F_{0.05}$; Reject the null hypothesis $(H_{1\ a})$

Hence, there are significantly difference in titers level of immunized mice both among tetanus toxoid preparations and periods of time.

Duncan's New Multiple Range Test for treatment (Data from table 38)

$S_x = \sqrt{0.1822/4} = 0.2134$

df of error = 18

P value	2	3	4	5	6	7
SSR	2.07	3.12	3.21	3.27	3.32	3.35
$LSR=SSR*S_x$	0.4418	0.6659	0.6851	0.6979	0.7086	0.7150

Preparation	TTMA	TT	TT+TTMC	TTMC	TT+TTMA	TT+TTMB	TTMB
X	1.25	1.66	2.06	2.30	2.95	3.03	3.15

TTMB,TTMA	3.15-1.252	=	1.908	>	0.7150	S
TTMB,TT	3.15-1.66	=	1.49	>	0.7086	S
TTMB,TT+TTMC	3.15-2.06	=	1.09	>	0.6979	S
TTMB,TTMC	3.15-2.31	=	0.84	>	0.6851	S
TTMB,TT+TTMA	3.15-2.95	=	0.020	<	0.6659	NS
TTMB,TT+TTMB	3.15-3.025	=	0.125	<	0.4418	NS

TT+TTMB,TTMA	3.025-1.25	=	1.775	>	0.7086	S
TT+TTMB,TT	3.025-1.66	=	1.365	>	0.6979	S
TT+TTMB,TT+TTMC	3.025-2.06	=	0.965	>	0.6851	S
TT+TTMB,TTMC	3.025-2.31	=	0.715	>	0.6659	S
TT+TTMB,TT+TTMA	3.025-2.95	=	0.075	<	0.4418	NS
TT+TTMA,TTMA	2.95-1.25	=	1.70	>	0.6979	S
TT+TTMA,TT	2.95-1.66	=	1.29	>	0.6851	S
TT+TTMA,TT+TTMC	2.95-2.06	=	0.89	<	0.6659	NS
TT+TTMA,TTMC	2.95-2.31	=	0.64	>	0.4418	S
TTMC,TTMA	2.31-1.25	=	1.06	>	0.6851	S
TTMC,TT	2.31-1.66	=	0.654	<	0.6659	NS
TTMC,TT+TTMC	2.31-2.06	=	0.012	<	0.4418	NS
TT+TTMC,TTMA	2.06-1.25	=	0.81	>	0.6659	S
TT+TTMC,TT	2.06-1.66	=	0.40	<	0.4418	NS
TT,TTMA	1.66-1.25	=	0.410	<	0.4418	NS

Duncan's New Multiple Range Test for block (Data from table 38)

$S_{\bar{x}} = \sqrt{0.1822/7} = 0.1613$

df of error = 18

P value	2	3	4
SSR	2.07	3.12	3.21
LSR=SSR×$S_{\bar{x}}$	0.3340	0.5034	0.5179

DAYS	90	75	60	45
X	1.79	2.39	2.64	2.56

Days 45,90 = 2.56 - 1.79 = 0.77 > 0.5179 S

45,75 = 2.56 - 2.39 = 0.17 < 0.5034 NS

45,60 = 2.56 - 2.640 = 0.08 < 0.3340 NS

60,90 = 2.64 - 1.79 = 0.85 > 0.5034 S

60,75 = 2.64 - 2.39 = 0.25 < 0.3340 NS

75,90 = 2.39 - 1.79 = 0.60 > 0.3340 S

Data from table 39

ΣX^2_{ij} = 50.8657

CT = $\frac{(\Sigma X_i^2)}{N}$-CT = $\frac{(35.59)^2}{35}$= 36.1899

SS_{total} = ΣX_{ij}^2-CT = 50.8657-36.1899 = 14.6755

SS_{block} = $\frac{(\Sigma X_i^2)}{n_i}$-CT = $\frac{278.92}{7}$-36.1899 = 3.6558

$SS_{treatment}$ = $\frac{\Sigma(\Sigma X^2_{.j})}{n_j}$-CT = $\frac{225.5801}{5}$ - 36.1899 = 8.9261

Table 42 ANOVA table of Mice's titer during day$ 105-180

Source	df	SS	MS	F
Treatment	6	8.9261	1.4877	17.0608
Block	4	3.6558	0.9139	10.4811
Residual	24	2.0938	0.0872	
Total	34	14.6757		

Treament; from table 43 , $F_{0.05}(6,24)$ = 2.49

17.0607> $F_{0.05}$; Reject the null hypothesis ($H_{1\ 0}$)

Block; from table 43 , $F_{0.05}$ (4,24) = 2.75

10.4811 > $F_{0.05}$; Reject the null hypothesis ($H_{1\ 0}$)

Hence, there are significantly difference in titers level of mice both among tetanus toxoid preparations and periods of time.

Duncan's New Multiple Range Test for treatment (Data from table 39)

$S_{\bar{x}} = \sqrt{0.0872/5} = 0.13219$

df of error = 24

P value	2	3	4	5	6	7
SSR	2.92	3.07	3.15	3.22	3.28	3.31
LSR=SSR×$S_{\bar{x}}$	0.3857	0.4055	0.4161	0.4254	0.4333	0.4373

Preparation	TT	TT+TTMA	TT+TTMC	TTMC	TTMA	TT+TTMB	TTMB
X	0.102	0.736	0.736	0.910	1.096	1.62	1.81

TTMB,TT = 1.81-0.102 =1.708 > 0.4373 S

TTMB,TT+TTMA = 1.81-0.736 =1.074 > 0.4254 S

TTMB,TT+TTMC = 1.81-0.736 =1.074 > 0.4254 S

TTMB+TTMC = 1.81-0.910 =0.90 > 0.4161 S

TTMB,TTMA = 1.81-1.096 =0.714 > 0.4056 S

TTMB,TT+TTMB =	1.81-1.62	=0.19	< 0.3857	NS
TT+TTMB,TT =	1.62-0.102	=1.518	> 0.4333	S
TT+TTMB,TT+TTMA	1.62-0.736	=0.884	> 0.4254	S
TT+TTMB,TT+TTMC	1.62-0.736	=0.884	> 0.4161	S
TT+TTMB,TTMC	1.62-0.910	=0.7100	> 0.4055	S
TTMA,TT =	1.096-0.102	= 0.994	> 0.4254	S
TTMA,TT+TTMA =	1.096-0.736	= 0.360	< 0.4161	NS
TTMA,TT+TTMC =	1.096-0.736	= 0.36	< 0.4055	NS
TTMA,TTMC =	1.096-0.910	= 0.186	< 0.3857	NS
TTMC,TT =	0.91-0.102	= 0.808	> 0.4161	S
TTMC,TT+TTMA =	0.91-0.736	= 0.174	< 0.4055	NS
TTMC,TT+TTMC =	0.91-0.736	= 0.174	< 0.3857	NS
TT+TTMC,TT =	0.736-0.102	= 0.634	> 0.4055	S
TT+TTMC,T+TTMA =	0.736-0.736	= 0	< 0.3857	NS
TT+TTMA,TT =	0.736-0.102	= 0.634	> 0.3857	S

Duncan's New Multiple Range Test for block (Data from table 39)

$S_{\bar{x}} = \sqrt{0.0872/7} = 0.1116$

df of error = 24

P value	2	3	4	5
SSR	2.92	3.07	3.15	3.22
LSR=SSR*$S_{\bar{x}}$	0.3259	0.3426	0.3515	0.3594

DAYS	160	180	140	120	105
X	0.67	0.68	0.86	1.15	1.65

Days 105,160 = 1.65-0.67 = 0.98 > 0.3426 S

105,180 = 1.65-0.68 = 0.97 > 0.3515 S

105,140 = 1.65-0.86 = 0.79 > 0.3426 S

105,120 = 1.65-1.15 = 0.57 > 0.3259 S

120,160 = 1.15-0.67 = 0.48 > 0.3515 S

120,180 = 1.15-0.68 = 0.47 > 0.3426 S

120,140 = 1.15-0.86 = 0.29 < 0.3259 NS

140,160 = 0.86-0.68 = 0.18 < 0.3426 NS

140,180 = 0.86-0.67 = 0.19 < 0.3259 NS

180,160 = 0.68-0.67 = 0.01 < 0.3259 NS

Table 43

F - Ratio for .05 (Above) and .01 (Below) Level of Significance

df2 \ df1	1	2	3	4	5	6	8	12	24	∞
1	161.45 4052.10	199.50 4999.03	215.72 5407.49	224.57 5625.14	230.17 5764.08	233.97 5829.39	238.89 5981.34	243.91 6105.83	249.04 6234.83	254.32 6366.48
2	18.51 98.49	19.00 99.01	19.16 99.17	19.25 99.25	19.30 99.30	19.33 99.33	19.37 99.36	19.41 99.42	19.45 99.46	19.50 99.50
3	10.13 34.12	9.55 30.81	9.28 29.46	9.12 28.71	9.01 28.24	8.94 27.91	8.84 27.49	8.74 27.05	8.64 26.60	8.53 26.12
4	7.71 21.20	6.94 18.00	6.59 16.69	6.39 15.98	6.26 15.52	6.16 15.21	6.04 14.80	5.91 14.37	5.77 13.93	5.63 13.46
5	6.61 16.26	5.79 13.27	5.41 12.06	5.19 11.39	5.05 10.97	4.95 10.67	4.82 10.27	4.68 9.89	4.53 9.47	4.36 9.02
6	5.99 13.74	5.14 10.92	4.76 9.78	4.53 9.15	4.39 8.75	4.28 8.47	4.15 8.10	4.00 7.72	3.84 7.31	3.67 6.88
7	5.59 12.25	4.74 9.55	4.35 8.45	4.12 7.85	3.97 7.46	3.87 7.19	3.73 6.84	3.57 6.47	3.41 6.07	3.23 5.65
8	5.32 11.26	4.46 8.65	4.07 7.59	3.84 7.01	3.69 6.63	3.58 6.37	3.44 6.03	3.28 5.67	3.12 5.28	2.93 4.86
9	5.12 10.56	4.26 8.02	3.86 6.99	3.63 6.42	3.48 6.06	3.37 5.80	3.23 5.47	3.07 5.11	2.90 4.73	2.71 4.31
10	4.96 10.04	4.10 7.56	3.71 6.55	3.48 5.99	3.33 5.64	3.22 5.39	3.07 3.06	2.91 4.71	2.74 4.33	2.54 3.91
11	4.84 9.65	3.98 7.20	3.59 6.22	3.36 5.67	3.20 5.32	3.09 5.07	2.95 4.74	2.79 4.40	2.61 4.02	2.40 3.60
12	4.75 9.33	3.88 6.93	3.49 5.95	3.26 3.41	3.11 5.06	3.00 4.82	2.85 4.50	2.69 4.16	2.50 3.78	2.30 3.36
15	4.54 8.63	3.68 6.36	3.29 5.42	3.06 4.89	2.79 4.56	2.64 4.32	2.48 4.00	2.29 3.67	2.39 3.29	2.07 2.87
20	4.35 8.10	3.49 5.85	3.10 4.94	2.87 4.43	2.71 4.10	2.60 3.87	2.45 3.56	2.28 3.23	2.08 2.36	1.84 2.42
25	4.24 7.77	3.38 5.57	2.99 4.68	2.76 4.18	2.60 3.86	2.49 3.63	2.34 3.32	2.16 2.99	1.96 2.62	1.71 2.17
30	4.17 7.56	3.32 5.39	2.92 4.51	2.69 4.02	2.53 3.70	2.42 3.47	2.27 3.17	2.09 2.84	1.89 1.47	1.62 2.01
∞	3.84 6.64	2.99 4.60	2.60 3.78	2.37 3.32	2.21 3.02	2.09 2.80	1.94 2.51	1.75 2.18	1.52 1.79	

Table 44

Significant Studentized Ranges for 5% and 1% Level New Multiple-range Test

Error df	Protection level	p = number of means for range being tested													
		2	3	4	5	6	7	8	9	10	11	14	16	18	20
1	.05	18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.0	18.0
	.01	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0	90.0
2	.05	6.09	6.09	6.09	6.09	6.09	6.09	6.09	6.09	6.09	6.09	6.09	6.09	6.09	6.09
	.01	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0
3	.05	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50	4.50
	.01	8.26	8.5	8.6	8.7	8.8	8.9	8.9	9.0	9.0	9.0	9.1	9.2	9.3	9.3
4	.05	3.93	4.01	4.02	4.02	4.02	4.02	4.02	4.02	4.02	4.02	4.02	4.02	4.02	4.02
	.01	6.51	6.8	6.9	7.0	7.1	7.1	7.2	7.2	7.3	7.3	7.4	7.4	7.5	7.5
5	.05	3.64	3.74	3.79	3.83	3.83	3.83	3.83	3.83	3.83	3.83	3.83	3.83	3.83	3.83
	.01	5.70	5.96	6.11	6.18	6.26	6.33	6.40	6.44	6.5	6.6	6.6	6.7	6.7	6.8
6	.05	3.46	3.58	3.64	3.68	3.68	3.68	3.68	3.68	3.68	3.68	3.68	3.68	3.68	3.68
	.01	5.24	5.51	5.65	5.73	5.81	5.88	5.95	6.00	6.0	6.1	6.2	6.2	6.3	6.3
7	.05	3.35	3.47	3.54	3.58	3.60	3.61	3.61	3.61	3.61	3.61	3.61	3.61	3.61	3.61
	.01	4.95	5.22	5.37	5.45	5.53	5.61	5.69	5.73	5.8	5.8	5.9	5.9	6.0	6.0
8	.05	3.26	3.39	3.47	3.52	3.55	3.56	3.56	3.56	3.56	3.56	3.56	3.56	3.56	3.56
	.01	4.74	5.00	5.14	5.23	5.32	5.40	5.47	5.51	5.5	5.6	5.7	5.7	5.8	5.8
9	.05	3.20	3.34	3.41	3.47	3.50	3.52	3.52	3.52	3.52	3.52	3.52	3.52	3.52	3.52
	.01	4.60	4.86	4.99	5.08	5.17	5.25	5.32	5.36	5.4	5.5	5.5	5.6	5.7	5.7
10	.05	3.15	3.30	3.37	3.43	3.46	3.47	3.47	3.47	3.47	3.47	3.47	3.47	3.47	3.48
	.01	4.48	4.73	4.88	4.96	5.06	5.13	5.20	5.24	5.28	5.36	5.42	5.48	5.54	5.55
11	.05	3.11	3.27	3.35	3.39	3.43	3.44	3.45	3.46	3.46	3.46	3.46	3.46	3.47	3.48
	.01	4.39	4.63	4.77	4.86	4.94	5.01	5.06	5.12	5.15	5.24	5.28	5.34	5.38	5.39
12	.05	3.08	3.23	3.33	3.36	3.40	3.42	3.44	3.44	3.46	3.46	3.46	3.46	3.47	3.48
	.01	4.32	4.55	4.68	4.76	4.81	4.92	4.96	5.02	5.07	5.13	5.17	5.22	5.24	5.26
13	.05	3.06	3.21	3.30	3.35	3.39	3.41	3.42	3.44	3.45	3.45	3.46	3.46	3.47	3.47
	.01	4.26	4.48	4.62	4.69	4.74	4.84	4.88	4.94	4.98	5.04	5.08	5.13	5.14	5.15
14	.05	3.03	3.18	3.27	3.33	3.37	3.39	3.41	3.42	3.44	3.45	3.46	3.46	3.47	3.47
	.01	4.21	4.42	4.55	4.63	4.70	4.78	4.83	4.87	4.91	4.96	5.00	5.04	5.06	5.07
15	.05	3.01	3.16	3.25	3.31	3.36	3.38	3.40	3.42	3.43	3.44	3.45	3.46	3.47	3.47
	.01	4.17	4.37	4.50	4.58	4.64	4.72	4.77	4.81	4.84	4.90	4.94	4.97	4.99	5.00
16	.05	3.00	3.15	3.23	3.30	3.34	3.37	3.39	3.41	3.43	3.44	3.45	3.46	3.47	3.47
17	.05	2.98	3.13	3.22	3.28	3.33	3.36	3.38	3.40	3.42	3.44	3.45	3.46	3.47	3.47
18	.05	2.07	3.12	3.21	3.27	3.32	3.35	3.37	3.39	3.41	3.43	3.45	3.46	3.47	3.47
19	.05	2.96	3.11	3.19	3.26	3.31	3.35	3.37	3.39	3.41	3.43	3.44	3.46	3.47	3.47
20	.05	2.95	3.10	3.18	3.25	3.30	3.34	3.36	3.38	3.40	3.43	3.44	3.46	3.46	3.47
22	.05	2.93	3.08	3.17	3.24	3.29	3.32	3.35	3.37	3.39	3.42	3.44	3.45	3.46	3.47
24	.05	2.92	3.07	3.15	3.22	3.28	3.31	3.34	3.37	3.38	3.41	3.44	3.45	3.46	3.47
26	.05	2.91	3.06	3.14	3.21	3.27	3.30	3.34	3.36	3.38	3.41	3.43	3.45	3.46	3.47
28	.05	2.90	3.04	3.13	3.20	3.26	3.30	3.33	3.35	3.37	3.40	3.43	3.45	3.46	3.47
30	.05	2.89	3.04	3.12	3.20	3.25	3.29	3.32	3.35	3.37	3.40	3.43	3.44	3.46	3.47
40	.05	2.86	3.01	3.10	3.17	3.22	3.27	3.30	3.33	3.35	3.39	3.42	3.44	3.46	3.47
60	.05	2.83	2.98	3.08	3.14	3.20	3.24	3.28	3.31	3.33	3.37	3.40	3.43	3.45	3.47
100	.05	2.80	2.95	3.05	3.12	3.18	3.22	3.26	3.29	3.32	3.36	3.40	3.42	3.45	3.47
∞	.05	2.77	2.92	3.02	3.09	3.15	3.19	3.23	3.26	3.29	3.34	3.38	3.41	3.44	3.47

Source: Abridged from D.B. Duncan, "Multiple range and multiple F tests," Biometrics. 11 : 1-42 (1955)

Appendix V

Titers in mice that immunized with TT

	Titers(units/ml)									
	Number of immunized mice									
Days	1	2	3	4	5	6	7	8	9	10
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
7	1.00	1.00	1.00	0.50	0.50	0.50	0.50	0.50	0.50	0.50
15	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
30	1.00	1.00	1.00	1.00	1.00	1.00	2.00	2.00	2.00	2.00
45	1.00	4.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
60	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
75	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
90	1.00	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
105	0.25	0.25	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
120	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
140	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
160	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
180	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Mean titers (unit/ml)in mice that immunized with TT

Days	ΣX	X	SD
0	0.00	0.00	0.00
3	1.30	0.13	0.00
7	6.50	0.65	0.24
15	10.00	1.00	0.00
30	14.00	1.40	0.52
45	21.00	2.10	0.74
60	20.00	2.00	0.00
75	20.00	2.00	0.00
90	5.50	0.55	0.16
105	4.50	0.45	0.23
120	0.60	0.06	0.00
140	0.00	0.00	0.00
160	0.00	0.00	0.00
180	0.00	0.00	0.00

Titers in mice that immunized with TTMA

	Titers(units/ml)									
	Number of immunized mice									
Days	1	2	3	4	5	6	7	8	9	10
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
30	0.06	0.06	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
45	0.25	0.25	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
60	1.00	1.00	1.00	1.00	1.00	0.50	0.50	0.50	0.50	0.50
75	1.00	1.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
90	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
105	1.00	1.00	1.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
120	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	2.00	2.00
140	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.50
160	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	0.50
180	1.00	1.00	1.00	1.00	0.50	0.50	0.50	0.50	0.50	0.50

Mean titers (Unit/ml)in mice that immunized with TTMA

Days	ΣX	X	SD
0	0.00	0.00	0.00
3	0.00	0.00	0.00
7	0.00	0.00	0.00
15	0.60	0.06	0.00
30	1.00	0.10	0.08
45	4.50	0.45	0.11
60	7.50	0.75	0.19
75	18.00	1.80	0.42
90	20.00	2.00	0.00
105	17.00	1.70	0.48
120	12.00	1.20	0.42
140	9.50	0.95	0.16
160	8.50	0.85	0.24
180	7.80	0.78	0.26

Titers in mice that immunized with TTMB

	Titers(units/ml)									
	Number of immunized mice									
Days	1	2	3	4	5	6	7	8	9	10
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
7	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
15	0.00	0.50	0.50	0.50	0.50	0.50	1.00	1.00	1.00	1.00
30	0.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
45	2.00	2.00	2.00	2.00	2.00	2.00	2.00	4.00	4.00	4.00
60	2.00	2.00	2.00	2.00	4.00	4.00	4.00	4.00	4.00	4.00
75	2.00	2.00	2.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
90	2.00	2.00	2.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
105	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	4.00	4.00
120	1.00	1.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	4.00
140	0.50	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
160	1.00	1.00	1.00	1.00	0.50	2.00	2.00	2.00	2.00	2.00
180	2.00	2.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Mean titers (Unit/ml)in mice that immunized with TTMB

Days	ΣX	X	SD
0	0.00	0.00	0.00
3	0.00	0.00	0.00
7	2.50	0.25	0.00
15	6.50	0.65	0.34
30	18.00	1.80	0.63
45	26.00	2.60	0.97
60	32.00	3.20	1.03
75	34.00	3.40	0.97
90	34.00	3.40	0.97
105	24.00	2.40	0.84
120	21.00	2.10	0.46
140	18.50	1.85	0.47
160	15.00	1.50	0.41
180	12.00	1.20	0.42

Titers in mice that immunized with TTMC

	Titers(units/ml)									
	Number of immunized mice									
Days	1	2	3	4	5	6	7	8	9	10
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.13	0.13	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
7	0.00	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
15	1.00	1.00	2.00	0.50	0.50	0.50	0.50	0.50	0.50	0.50
30	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
45	0.00	2.00	2.00	2.00	2.00	4.00	4.00	4.00	4.00	4.00
60	2.00	2.00	2.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
75	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
90	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
105	1.00	1.00	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
120	0.00	0.50	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
140	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.25	0.25
160	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
180	0.25	0.25	0.25	0.25	0.50	0.50	0.50	0.50	0.50	0.50

Mean titers (Unit/ml)in mice that immunized with TTMC

Days	ΣX	X	SD
0	0.00	0.00	0.00
3	0.74	0.07	0.03
7	4.50	0.45	0.16
15	7.50	0.75	0.49
30	20.00	2.00	0.00
45	28.00	2.80	1.40
60	34.00	3.40	0.97
75	10.00	1.00	0.00
90	10.00	1.00	0.00
105	6.00	0.60	0.21
120	2.50	0.25	0.12
140	4.50	0.45	0.11
160	4.00	0.40	0.32
180	4.00	0.40	0.13

Titers in mice that immunized with TT+TTMA

	Titers(units/ml)									
	Number of immunized mice									
Days	1	2	3	4	5	6	7	8	9	10
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.06	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
7	0.50	0.50	0.50	0.50	1.00	1.00	1.00	1.00	1.00	1.00
15	2.00	2.00	2.00	2.00	2.00	2.00	1.00	1.00	1.00	1.00
30	2.00	2.00	2.00	2.00	2.00	2.00	2.00	4.00	4.00	4.00
45	2.00	2.00	2.00	2.00	4.00	4.00	4.00	4.00	4.00	4.00
60	2.00	2.00	2.00	2.00	4.00	4.00	4.00	4.00	4.00	4.00
75	2.00	2.00	2.00	2.00	4.00	4.00	4.00	4.00	4.00	4.00
90	4.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
105	1.00	1.00	1.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
120	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	2.00
140	0.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
160	0.50	0.50	0.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00
180	0.50	0.50	0.50	0.50	1.00	1.00	1.00	1.00	1.00	1.00

Mean titers (Unit/ml)in mice that immunized with TT+TTMA

Days	ΣX	$\overline{X}$	SD
0	0.00	0.00	0.00
3	1.50	0.15	0.02
7	8.00	0.80	0.26
15	16.00	0.16	0.52
30	26.00	2.60	0.97
45	32.00	3.20	1.03
60	32.00	3.20	1.03
75	32.00	3.20	1.03
90	22.00	2.20	0.63
105	17.00	1.70	0.48
120	11.00	1.10	0.32
140	9.50	0.95	0.16
160	8.50	0.85	0.35
180	8.00	0.80	0.26

Titers in mice that immunized with TT+TTMB

	Titers(units/ml)									
	Number of immunized mice									
Days	1	2	3	4	5	6	7	8	9	10
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.06	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
7	0.50	0.50	0.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00
15	1.00	1.00	1.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
30	4.00	4.00	4.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
45	2.00	2.00	2.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
60	2.00	2.00	2.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00
75	2.00	2.00	2.00	2.00	4.00	0.00	4.00	4.00	4.00	4.00
90	1.00	2.00	2.00	2.00	2.00	2.00	2.00	4.00	4.00	4.00
105	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	4.00
120	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	1.00	1.00
140	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	2.00	2.00
160	2.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
180	2.00	2.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

Mean titers (Unit/ml)in mice that immunized with TT+TTMB

Days	ΣX	$\bar{X}$	SD
0	0.00	0.00	0.00
3	1.20	0.12	0.08
7	8.50	0.85	0.24
15	16.00	1.60	0.48
30	26.00	2.60	0.70
45	34.00	3.40	0.97
60	34.00	3.40	0.97
75	28.00	2.80	1.03
90	25.00	2.50	1.08
105	22.00	2.20	0.63
120	19.00	1.90	0.48
140	14.00	1.40	0.63
160	11.00	1.10	0.32
180	12.00	1.20	0.46

Titers in mice that immunized with TT+TTMC

	Titers(units/ml)									
	Number of immunized mice									
Days	1	2	3	4	5	6	7	8	9	10
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.25	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
7	0.50	0.50	0.50	0.50	1.00	1.00	1.00	1.00	1.00	1.00
15	1.00	1.00	1.00	1.00	2.00	2.00	2.00	2.00	2.00	2.00
30	4.00	4.00	4.00	4.00	4.00	2.00	2.00	2.00	2.00	0.00
45	4.00	4.00	4.00	4.00	4.00	4.00	4.00	2.00	2.00	2.00
60	4.00	4.00	4.00	2.00	2.00	2.00	2.00	2.00	2.00	1.00
75	1.00	1.00	0.50	0.50	2.00	2.00	2.00	2.00	2.00	2.00
90	0.50	0.50	0.50	1.00	1.00	1.00	1.00	1.00	1.00	1.00
105	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
120	0.00	0.00	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
140	0.00	0.00	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
160	0.00	0.00	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
180	0.00	0.00	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25

Mean titers (Unit/ml)in mice that immunized with TT+TTMC

Days	ΣX	$\overline{X}$	SD
0	0.00	0.00	0.00
3	1.50	0.15	0.04
7	8.00	0.80	0.26
15	16.00	1.60	0.52
30	28.00	2.80	1.40
45	34.00	3.40	0.97
60	25.00	2.50	1.08
75	15.00	1.50	0.67
90	8.50	0.85	0.24
105	5.00	0.50	0.00
120	4.00	0.40	0.21
140	4.00	0.40	0.21
160	2.00	0.20	0.11
180	2.00	0.20	0.11

Titers in mice that immunized with PBS pH 7.4

	Titers(units/ml)									
	Number of immunized mice									
Days	1	2	3	4	5	6	7	8	9	10
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
30	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
45	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
60	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
75	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
90	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
105	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
140	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
160	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
180	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Appendix VII

List of Abbrevations

ANOVA	=	Analysis of varience
A	=	Angstom
C	=	Degree celcious
g.	=	Gram
mg.	=	Milligram
min	=	minutes
ml.	=	Millilitre
mm.	=	Millimetre
no.	=	Quantity
NS	=	Non significantly difference
rpm	=	Round per minutes
S	=	Significantly difference
um	=	Micrometre
u/ml	=	Unit/milliliters.
%	=	Percentage

Curriculum Vitae

Mrs. Suchada Natesuwon was born on May 31,1962 in Songkhla, Thailand. She received her Bachelor of Science in Pharmacy in 1985 from the Faculty of Pharmacy, Prince of Songkhla University and Bachelor of General Managemenr in Business Administration in 1990 from the Faculty of Business Administration , Sukhothai Thammathirat University. She presents as a pharmacist at The Thai Red Cross Society, Science Division.