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Appendix I

Bleeding Method

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 bottom 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 (shortbovel) needle attached to a 2 ml. syringe is inserted between the left intercostal spaçes 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 When blood appears in the barrel of the syringe, the piston should be withdrawn very slowly, since too great negative pressure tends to collapse the heart wall.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 exact 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 with 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 While the heart still beats, the pipette is introduced into the wall of the left ventricle, and blood is withdrawn slowly.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.

Preparation of Serum from Blood

In most immunological procedure serum is employed in preference to plasma. Serum is obtained by allowing blood to (lot., 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 with further incubation either in the water bath or by overnight storage in the cold.

Appendix II

Statistical Analysis

a) Potency testing

1) Result from table 3 were filled in contingency table 28, 30, and 32

Table 28 Contingency table of survived mice during day 0 and 15.

Day	TT	TTM	TT+TTM	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
0	0	0	0	0	0	0
3	0	0	0	0	0	0
7	5	0	5	10	100	3.33
15	9	5	8	22	484	7.33
Ex_i	14	5	13	32		
$(Ex_i)^2$	196	25	169			
$\overline{x_i}$	3.50	1.25	3.25			

Hypothesis

Treatment

H_{1O} : There are no significant difference in number of survived mice among tetanus toxoid preparation.

H_{1A} : There are significant difference in number of survived mice among tetanus toxoid preparation.

Block

H_{2O} : There are no significant difference in number of survived mice at each time period.

H_{2A} : There are significant difference in number of survived mice at each time period.

Calculation data from table 28

$Ex_{ij}^{2} \quad = \quad 220$

$$\text{Correction Term (CT)} = \frac{(E_{ij}\, x_{ij})^{2}}{N} = \frac{(32)^{2}}{(4)(3)} = 85.3333$$

$$\text{SS.Total} = Ex_{ij}^{2} - CT$$

$$= 220 - 85.3333$$

$$= 134.6667$$

$$\text{SS.Block} = \frac{E\,(Ex_{j})^{2}}{nj} - CT$$

$$= \frac{(0 + 0 + 100 + 484)}{3} - 85.3333$$

$$= 109.333$$

$$\text{SS.Treatmetn} = \frac{E(Ex_{i})^{2}}{ni} - CT$$

$$= \frac{(196 + 25 + 169)}{4} - 85.3333$$

$$= 12.1667$$

Table 29 ANOVA table of survived mice during day 0 and 15.

Source	df	SS.	MS	F
Treatment	2	12.1667	6.0834	2.77
Block	3	109.3333	36.4444	16.61
Residual	6	13.1667	2.1945	
Total	11	134.6667		

degree of freedom of total = (3) 4) - 1 = 11

degree of freedom of block = 4 - 1 = 3

degree of freedom of treatment = 3 - 1 = 2

degree of freedom of residual = (3-1) (4-1) = 6

Treatment ; from table, $F_{0.05}$ (2, 6) = 5.14

2.77 < $F_{0.05}$; Accept the null hypothesis (H_{10}) (P<0.05)

Block ; from table, $F_{0.05}$ (3, 6) = 4.76

16.61 > $F_{0.05}$; Reject the null hypothesis (H_{20}) (P>0.05)

Hence, there are no significant difference in number of survived mice among tetanus toxoid preparation but there are significant difference in number of survived mice at each time period.

Duncan's New Multiple Range Test for block (Data from table 28)

$Sx = \sqrt{2.1945/3} = 0.8553$

df of error = 6

P value	2	3	4
SSR	3.46	3.58	3.64
LSR = (SSR) Sx	2.9593	3.0620	3.1133

Day	0	3	7	15
X	0	0	3.33	7.33

Day	15, 0	=	7.33 - 0	=	7.33 > 3.1133	S
Day	15, 3	=	7.33 - 0	=	7.33 > 3.0620	S
Day	15, 7	=	7.33 - 3.33	=	4.00 > 2.9593	S
Day	7, 0	=	7.33 - 0	=	3.33 > 3.0620	S
Day	7, 3	=	3.33 - 0	=	3.33 > 2.9593	S
Day	3, 0	=	0 - 0	=	0 < 2.9529	NS

Table 30 Contingency table of survived mice during day 30 and 75.

Day	TT	TTM	TT+TTM	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
30	10	9	10	29	841	9.67
45	10	10	10	30	900	10.00
60	10	10	10	30	900	10.00
75	8	10	10	28	784	9.33
Ex_i	38	39	40	117		
$(Ex_i^2$	1444	1521	1600			
$\overline{x_i}$	9.50	9.75	10			

Calculation data from table 30

$Ex_{ij}^2 = 1145$

Correction Term (CT) $= \dfrac{(E_{ij}\, x_{ij})^2}{N} = \dfrac{(117)^2}{(4)(3)} = 1140.75$

SS.Total $= Ex_{ij}^2 - CT$

$= 1145 - 1140.75 = 4.25$

SS.Block $= \dfrac{E\,(Ex_j)^2}{nj} - CT$

$= \dfrac{(841 + 900 + 900 + 784)}{3} - 1140.75 = 0.9167$

SS.Treatment $= \dfrac{E(Ex_i)^2}{ni} - CT$

$= \dfrac{(1444 + 1521 + 1600)}{4} - 1140.75 = 0.5000$

Table 31 ANOVA table of survived mice during day 30 and 75.

Source	df	SS.	MS	F
Treatment	2	0.5000	0.2500	0.5294
Block	3	0.9167	0.3056	0.3334
Residual	6	2.8333	0.4722	
Total	11	4.2500		

degree of freedom of total = (3) 4) - 1 = 11

degree of freedom of block = 4 - 1 = 3

degree of freedom of treatment = 3 - 1 = 2

degree of freedom of residual = (3-1) (4-1) = 6

Treatment ; from table, $F_{0.05}$ (2, 6) = 5.14

0.5294 < $F_{0.05}$; Accept the null hypothesis (H_{10}) (P<0.05)

Block ; from table, $F_{0.05}$ (3, 6) = 4.76

0.3334 < $F_{0.05}$; Accept the null hypothesis (H_{20}) (P<0.05)

Hence, there are no significant difference in number of survived mice both among tetanus toxoid preparation and each time period.

Table 32 Contingency table of survived mice during day 90 and 180.

Day	TT	TTM	TT+TTM	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
90	5	10	10	25	625	8.33
120	0	10	10	20	400	6.67
150	0	10	10	20	400	6.67
180	0	10	10	20	400	6.67
Ex_i	5	40	40	85		
$(Ex_i^2$	25	1600	1600			
$\overline{x_i}$	1.25	10	10			

Data from table 32

$Ex_{ij}^2 \quad = \quad 825$

Correction Term (CT) $= \frac{(E_{ij}\, x_{ij})^2}{N} = \frac{(85)^2}{(4)(3)} = 602.0833$

SS.Total $= Ex_{ij}^2 - CT$

$= 825 - 602.0833$

$= 222.9167$

SS.Block $= \frac{E\,(Ex_j)^2}{nj} - CT$

$= \frac{(625 + 400 + 400 + 400)}{3} - 602.0833 = 6.2500$

SS.Treatment $= \frac{E(Ex_i)^2}{ni} - CT$

$= \frac{(25 + 1600 + 1600)}{4} - 602.0833 = 204.1667$

Table 33 ANOVA table of survived mice during day 90 and 180.

Source	df	SS.	MS	F
Treatment	2	204.1667	102.0834	49.0008
Block	3	6.2500	2.0833	1.0000
Residual	6	12.5000	2.0833	
Total	11	222.9167		

Treatment ; from table, $F_{0.05}$ (2, 6) = 5.14

49.0008 > $F_{0.05}$; Reiect the null hypothesis (H_{10}) (P>0.05)

Block ; from table, $F_{0.05}$ (3, 6) = 4.76

1.0000 < $F_{0.05}$; Accept the null hypothesis (H_{20}) (P<0.05)

Hence, there are significant difference in number of survived mice among tetanus toxoid preparation but there are no significant difference in number of survived mice at each time period.

Duncan's New Multiple Range Test for treatment (Data from table 32)

$Sx = \sqrt{2.0833/4} = 0.7217$

df of error = 6

P value	2	3
SSR	3.46	3.58
LSR = (SSR) Sx	2.4971	2.25837

Preparation	TT	TTM	TT+TTM
X	1.25	10	10

TT + TTM, TT	=	10 - 1.25	= 8.75 > 2.5837	S
TT + TTM, TTM	=	10 - 10	= 0 < 2.4971	NS
TTM, TT	=	10 - 1.25	= 8.75 > 2.4971	S

2) Result from table 5 were filled in confingency table 34, 36, and 38

Table 34 Contingency table of survived mice during day 0 and 15.

Day	TT	TTM	TT+TTM	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
0	0	0	0	0	0	0
3	0	0	0	0	0	0
7	5	1	4	10	100	3.33
15	8	6	8	22	484	7.33
Ex_i	13	7	12	32		
$(Ex_i)^2$	169	49	144			
$\overline{x_i}$	3.25	1.75	3.0			

Calculation data from table 34

$$Ex_{ij}^2 = 206$$

$$\text{Correction Term (CT)} = \frac{(E_{ij}\,x_{ij})^2}{N} = \frac{(32)^2}{(4)(3)} = 85.3333$$

$$\text{SS.Total} = Ex_{ij}^2 - CT$$

$$= 206 - 85.3333 = 120.6667$$

$$\text{SS.Block} = \frac{E\,(Ex_j)^2}{nj} - CT$$

$$= \frac{(0 + 0 + 100 + 484)}{3} - 85.3333 = 109.3333$$

$$\text{SS.Treatment} = \frac{E(Ex_i)^2}{ni} - CT$$

$$= \frac{(169 + 49 + 144)}{4} - 85.3333 = 5.1667$$

Table 35 ANOVA table of survived mice during day 0 and 15.

Source	df	SS.	MS	F
Treatment	2	5.1667	2.5834	2.51
Block	3	109.3333	36.4444	35.46
Residual	6	6.1667	1.0278	
Total	11	120.6667		

Treatment ; from table, $F_{0.05}$ (2, 6) = 5.14

$2.51 < F_{0.05}$; Accept the null hypothesis (H_{10}) ($P<0.05$)

Block ; from table, $F_{0.05}$ (3, 6) = 4.76

$35.46 > F_{0.05}$; Reject the null hypothesis (H_{20}) ($P>0.05$)

Hence, there are no significant difference in number of survived mice among tetanus toxoid preparation but there are significant difference in number of survived mice at each time period.

Duncan's New Multiple Range Test for block (Data from table 34)

Sx = $\sqrt{1.0278/3}$ = 0.5853

df of error = 6

P value	2	3	4
SSR	3.46	3.58	3.64
LSR = (SSR) Sx	2.0251	2.0954	2.1305

Day	0	3	7	15
X	0	0	3.33	7.33

Day	15, 0	=	7.33 - 0	=	7.33 > 2.1305	S
Day	15, 3	=	7.33 - 0	=	7.33 > 2.0954	S
Day	15, 7	=	7.33 - 3.33	=	4.00 > 2.0251	S
Day	7, 0	=	3.33 - 0	=	3.33 > 2.0954	S
Day	7, 3	=	3.33 - 0	=	3.33 > 2.0251	S
Day	3, 0	=	0 - 0	=	0 < 2.0251	NS

Table 36 Contingency table of survived mice during day 30 and 75.

Day	TT	TTM	TT+TTM	Ex_j	$(Ex_j)^2$	$\overline{x}_j$
30	10	10	10	30	900	10
45	10	10	10	30	900	10
60	10	10	10	30	900	10
75	7	10	10	27	729	9
Ex_i	37	40	40	117		
$(Ex_i)^2$	1369	1600	1600			
$\overline{x}_i$	9.25	10	10			

Data from table 36

$Ex_{ij}^2 = 1149$

Correction Term (CT) $= \frac{(E_{ij}\, x_{ij})^2}{N} = \frac{(117)^2}{(4)(3)} = 1140.75$

SS.Total $= Ex_{ij}^2 - CT$

$= 1149 - 1140.75$

$= 8.25$

SS.Block $= \frac{E\,(Ex_j)^2}{nj} - CT$

$= \frac{(900 + 900 + 900 + 729)}{3} - 1140.75 = 2.25$

SS.Treatment $= \frac{E(Ex_i)^2}{ni} - CT$

$= \frac{(1369 + 1600 + 1600)}{4} - 1140.75 = 1.50$

Table 37 ANOVA table of survived mice during day 30 and 75.

Source	df	SS.	MS	F
Treatment	2	1.5000	0.7500	1.0000
Block	3	2.2500	0.7500	1.0000
Residual	6	4.5000	0.7500	
Total	11	8.2500		

Treatment ; from table, $F_{0.05}$ (2, 6) = 5.14

1.0000 < $F_{0.05}$; Accept the null hypothesis (H_{10}) (P<0.05)

Block ; from table, $F_{0.05}$ (3, 6) = 4.76

1.0000 < $F_{0.05}$; Accept the null hypothesis (H_{20}) (P<0.05)

Hence, there are no significant difference in number of survived mice both among tetanus toxoid preparation and each time period.

Table 38 Contingency table of survived mice during day 90 and 180.

Day	TT	TTM	TT+TTM	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
90	5	10	10	25	625	8.33
120	0	9	10	19	361	6.33
150	0	10	10	20	400	6.67
180	0	10	10	20	400	6.67
Ex_i	5	39	40	84		
$(Ex_i)2$	25	1521	1600			
$\overline{x_i}$	1.25	9.75	10.0			

Data from table 38

$Ex_{ij}^2 = 806$

Correction Term (CT) $= \frac{(E_{ij}\, x_{ij})^2}{N} = \frac{(84)^2}{(4)(3)} = 588.0000$

SS.Total $= Ex_{ij}^2 - CT$

$= 806 - 588.0000$

$= 218.0000$

SS.Block $= \frac{E\ (Ex_j)^2}{nj} - CT$

$= \frac{(625 + 361 + 400 + 400)}{3} - 588.0000 = 7.3333$

SS.Treatment $= \frac{E(Ex_i)^2}{ni} - CT$

$= \frac{(25 + 1521 + 1600)}{4} - 588.0000 = 198.5000$

Table 39 ANOVA table of survived mice during day 90 and 180.

Source	df	SS.	MS	F
Treatment	2	198.5000	99.25	48.9447
Block	3	7.3333	2.4444	1.2054
Residual	6	12.1667	2.0278	
Total	11	218.0000		

Treatment ; from table, $F_{0.05}$ (2, 6) = 5.14

48.9447 > $F_{0.05}$; Reject the null hypothesis (H_{10}) (P>0.05)

Block ; from table, $F_{0.05}$ (3, 6) = 4.76

1.2054 < $F_{0.05}$; Accept the null hypothesis (H_{20}) (P<0.05)

Hence, there are significant difference in number of survived mice among tetanus toxoid preparation but there are no significant difference in number of survived mice at each time period.

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

$Sx = \sqrt{2.0278/4} = 0.7120$

df of error = 6

P value	2	3
SSR	3.46	3.58
LSR = (SSR) Sx	2.4635	2.5489

Preparation	TT	TTM	TT+TTM
X	1.25	9.75	10.0

TT + TTM, TT	=	10 - 1.25	= 8.75 > 2.5489	S
TT + TTM, TTM	=	10 - 9.75	= 0.25 < 2.4635	NS
TTM, TT	=	9.75 - 1.25	= 8.50 > 2.4635	S

3) Result from table 7 were filled in contingency table 40, 42 and 44

Table 40 Contingency table of survived mice during day 0 and 15.

Day	TT	TTM	TT+TTM	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
0	0	0	0	0	0	0
3	0	0	0	0	0	0
7	5	0	6	11	121	3.67
15	9	7	9	25	625	8.33
Ex_i	14	7	15	36		
$(Ex_i)^2$	196	49	225			
$\overline{x_i}$	3.50	1.75	3.75			

Calculation data from table 40

$Ex_{ij}^2 = 272$

Correction Term (CT) $= \frac{(E_{ij}x_{ij})^2}{N} = \frac{(36)^2}{(4)(3)} = 108.0000$

SS.Total $= Ex_{ij}^2 - CT$

$= 272 - 108.0000 = 164.0000$

SS.Block $= \frac{E(Ex_j)^2}{nj} - CT$

$= \frac{(0 + 0 + 121 + 625)}{3} - 108.0000 = 140.6667$

SS.Treatment $= \frac{E(Ex_i)^2}{ni} - CT$

$= \frac{(196 + 49 + 225)}{4} - 108.0000 = 9.5000$

Table 41 ANOVA table of survived mice during day 0 and 15.

Source	df	SS.	MS	F
Treatment	2	9.5000	4.7500	2.0602
Block	3	140.6667	46.8889	20.3369
Residual	6	13.8333	2.3056	
Total	11	164.0000		

Treatment ; from table, $F_{0.05}$ (2, 6) = 5.14

2.0602 < $F_{0.05}$; Accept the null hypothesis (H_{10}) (P<0.05)

Block ; from table, $F_{0.05}$ (3, 6) = 4.76

20.3336 > $F_{0.05}$; Reject the null hypothesis (H_{20}) (P>0.05)

Hence, there are no significant difference in number of survived mice among tetanus toxoid preparation but there are significant difference in number of survived mice at each time period.

Duncan's New Multiple Range Test for block (Data from table 40)

$$Sx = \sqrt{2.3056/3} = 0.8767$$

df of error = 6

P value	2	3	4
SSR	3.46	3.58	3.64
LSR = (SSR) Sx	3.0333	3.1386	3.1912

Day	0	3	7	15
X	0	0	3.67	8.33

Day	15, 0	=	8.33 - 0	=	8.33 > 3.1912	S
Day	15, 3	=	8.33 - 0	=	8.33 > 3.1386	S
Day	15, 7	=	8.33 - 3.67	=	4.66 > 3.0333	S
Day	7, 0	=	3.67- 0	=	3.67 > 3.1386	S
Day	7, 3	=	3.67 - 0	=	3.67 > 3.0333	S
Day	3, 0	=	0 - 0	=	0 < 3.0333	NS

Table 42 Contingency table of survived mice during day 30 and 75.

Day	TT	TTM	TT+TTM	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
30	10	10	10	30	900	10
45	10	10	10	30	900	10
60	9	10	10	29	841	9.67
75	6	10	10	26	676	8.67
Ex_i	35	40	40	115		
$(Ex_i)^2$	1225	1600	1600			
$\overline{x_i}$	8.75	10	10			

Data from table42

$Ex_{ij}^2 = 1117$

Correction Term (CT) $= \frac{(E_{ij}\, x_{ij})^2}{N} = \frac{(115)^2}{(4)(3)} = 1102.0833$

SS.Total $= Ex_{ij}^2 - CT$

$= 1117 - 1102.0833 = 14.9167$

SS.Block $= \frac{E\,(Ex_j)^2}{nj} - CT$

$= \frac{(900 + 900 + 841 + 676)}{3} - 1102.0833 = 3.5834$

SS.Treatment $= \frac{E(Ex_i)}{ni} - CT$

$= \frac{(1225 + 1600 + 1600)}{4} - 1102.0833 = 4.1667$

Table 43 ANOVA table of survived mice during day 30 and 75.

Source	df	SS.	MS	F
Treatment	2	4.1667	2.0834	1.7443
Block	3	3.5834	1.1945	1.0001
Residual	6	7.1666	1.1944	
Total	11	14.9167		

Treatment ; from table, $F_{0.05}$ (2, 6) = 5.14

1.7443 < $F_{0.05}$; Accept the null hypothesis (H_{10}) (P<0.05)

Block ; from table, $F_{0.05}$ (3, 6) = 4.76

1.0001 < $F_{0.05}$; Accept the null hypothesis (H_{20}) (P<0.05)

Hence, there are no significant difference in number of survived mice both among tetanus toxoid preparation and each time period.

Table 44 Contingency table of survived mice during day 90 and 180.

Day	TT	TTM	TT+TTM	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
90	4	10	10	24	576	8.0
120	0	9	10	19	361	6.33
150	0	10	9	19	361	6.33
180	0	10	10	20	400	6.67
Ex_i	4	39	39	82		
$(Ex_i)^2$	16	1521	1521			
$\overline{x_i}$	1.0	9.75	9.75			

Data from table 44

$Ex_{ij}^2 = 778$

Correction Term (CT) $= \dfrac{(E_{ij}\, x_{ij})^2}{N} = \dfrac{(82)^2}{(4)(3)} = 560.3333$

SS.Total $= Ex_{ij}^2 - CT$

$= 778 - 560.3333$

$= 217.6667$

SS.Block $= \dfrac{E\,(Ex_j)^2}{nj} - CT$

$= \dfrac{(576 + 361 + 361 + 400)}{3} - 560.3333 = 5.6667$

SS.Treatment $= \dfrac{E(Ex_i)^2}{ni} - CT$

$= \dfrac{(16 + 1521 + 1521)}{4} - 560.3333 = 204.1667$

Table 45 ANOVA table of survived mice during day 90 and 180.

Source	df	SS.	MS	F
Treatment	2	204.1667	102.0834	78.1889
Block	3	5.6667	1.8889	1.4468
Residual	6	7.8333	1.3056	
Total	11	217.6667		

Treatment ; from table, $F_{0.05}$ (2, 6) = 5.14

78.1889 > $F_{0.05}$; Reject the null hypothesis (H_{10}) (P>0.05)

Block ; from table, $F_{0.05}$ (3, 6) = 4.76

1.4468 < $F_{0.05}$; Accept the null hypothesis (H_{20}) (P<0.05)

Hence, there are significant difference in number of survived mice among tetanus toxoid preparation but there are no significant difference in number of survived mice at each time period.

Duncan's New Multiple Range Test for Treatment (Data from table 44)

$Sx = \sqrt{1.3056/4} = 0.5713$

df of error = 6

P value	2	3
SSR	3.46	3.58
LSR = (SSR) Sx	1.9767	2.0453

Preparation	TT	TTM	TT+TTM
X	1.0	9.75	9.75

TT + TTM, TT	= 9.75 - 1.0	= 8.75 > 2.0453		S
TT + TTM, TTM	= 9.75 - 9.75	= 0 < 1.9767		NS
TTM, TT	= 9.75 - 1.0	= 8.75 > 1.9767		S

4) Result from table 9 were filled in contingency table 46, 48 and 50

<u>**Table 46**</u> Contingency table of survived mice during day 0 and 15.

Day	TT	TTM	TT+TTM	Ex_j	$(Ex_j)^2$	$\overline{x}_j$
0	0	0	0	0	0	0
3	0	0	0	0	0	0
7	4	2	5	11	121	3.67
15	8	6	8	22	484	7.33
Ex_i	12	8	13	33		
$Ex_i)^2$	144	64	169			
$\overline{x}_i$	3.0	2.0	3.25			

<u>**Calculation**</u> data from table 46

$Ex_{ij}^2 = 209$

Correction Term (CT) $= \frac{(E_{ij}\,x_{ij})^2}{N} = \frac{(33)^2}{(4)(3)} = 90.7500$

SS.Total $= Ex_{ij}^2 - CT$

$= 209 - 90.7500 = 118.2500$

SS.Block $= \frac{E\,(Ex_j)^2}{nj} - CT$

$= \frac{(0 + 0 + 121 + 484)}{3} - 90.7500 = 110.9167$

SS.Treatment $= \frac{E(Ex_i)^2}{ni} - CT$

$= \frac{(144 + 64 + 169)}{4} - 90.7500 = 3.5000$

Table 47 ANOVA table of survived mice during day 0 and 15.

Source	df	SS.	MS	F
Treatment	2	3.5000	1.7500	2.74
Block	3	110.9167	36.9722	57.87
Residual	6	3.8333	0.6389	
Total	11	118.2500		

Treatment ; from table, $F_{0.05}$ (2, 6) = 5.14

2.74 < $F_{0.05}$; Accept the null hypothesis (H_{10}) (P<0.05)

Block ; from table, $F_{0.05}$ (3, 6) = 4.76

57.87 > $F_{0.05}$; Reject the null hypothesis (H_{20}) (P>0.05)

Hence, there are no significant difference in number of survived mice among tetanus toxoid preparation but there are significant difference in number of survived mice at each time period.

Duncan's New Multiple Range Test for block (Data from table 46)

Sx = $\sqrt{0.6389/3}$ = 0.4615

df of error = 6

P value	2	3	4
SSR	3.46	3.58	3.64
LSR = (SSR) Sx	1.5968	1.6522	1.6799

Day	0	3	7	15
X	0	0	3.67	7.33

Day	15, 0	=	7.33 - 0	=	7.33 > 1.6799	S
Day	15, 3	=	7.33 - 0	=	7.33 > 1.6522	S
Day	15, 7	=	7.33 - 3.67	=	3.66 > 1.5968	S
Day	7, 0	=	3.67- 0	=	3.67 > 1.6522	S
Day	7, 3	=	3.67 - 0	=	3.67 > 1.5968	S
Day	3, 0	=	0 - 0	=	0 < 1.5968	NS

<u>Table 48</u> Contingency table of survived mice during day 30 and 75.

Day	TT	TTM	TT+TTM	Ex_j	$(Ex_j)^2$	$\overline{x}_j$
30	10	9	10	29	841	9.67
45	10	10	10	30	900	10
60	9	10	10	29	841	9.67
75	5	10	10	25	625	8.33
Ex_j	34	39	40	113		
$(Ex_j)^2$	1156	1521	1600			
$\overline{x}_j$	8.50	9.75	10			

<u>Data from table 48</u>

$Ex_{ij}^2 = 1087$

Correction Term (CT) $= \dfrac{(E_{ij}\,x_{ij})^2}{N} = \dfrac{(113)^2}{(4)(3)} = 1064.0833$

SS.Total $= Ex_{ij}^2 - CT$

$= 1087 - 1064.0833 = 22.9167$

SS.Block $= \dfrac{E\,(Ex_j)^2}{nj} - CT$

$= \dfrac{(84 + 900 + 841 + 625)}{3} - 1064.0833 = 4.9167$

SS.Treatment $= \dfrac{E(Ex_i)^2}{ni} - CT$

$= \dfrac{(1156 + 1521 + 1600)}{4} - 1064.0833 = 5.1667$

Table 49 ANOVA table of survived mice during day 30 and 75.

Source	df	SS.	MS	F
Treatment	2	5.1667	2.5834	1.2078
Block	3	4.9167	1.6389	0.7662
Residual	6	12.8333	2.1389	
Total	11	22.9167		

Treatment ; from table, $F_{0.05}$ (2, 6) = 5.14

1.2078 < $F'_{0.05}$; Accept the null hypothesis (H_{10}) (P<0.05)

Block ; from table, $F_{0.05}$ (3, 6) = 4.76

0.7662 < $F_{0.05}$; Accept the null hypothesis (H_{20}) (P<0.05)

Hence, there are no significant difference in number of survived mice both among tetanus toxoid preparation and each time period.

Table 50 Contingency table of survived mice during day 90 and 180.

Day	TT	TTM	TT+TTM	Ex_j	$(Ex_j)^2$	$\overline{x}_j$
90	3	9	10	22	484	7.33
120	0	10	10	20	400	6.67
150	0	10	10	20	400	6.67
180	0	10	10	20	400	6.67
Ex_j	3	39	40	82		
$(Ex_j)^2$	9	1521	1600			
$\overline{x}_j$	0.75	9.75	10			

Data from table 50

$Ex_{ij}^2 = 790$

Correction Term (CT) $= \dfrac{(E_{ij}\,x_{ij})^2}{N} = \dfrac{(82)^2}{(4)(3)} = 560.3333$

SS.Total $= Ex_{ij}^2 - CT$

$= 790 - 560.3333$

$= 229.6667$

SS.Block $= \dfrac{E\,(Ex_j)^2}{nj} - CT$

$= \dfrac{(484 + 400 + 400 + 400)}{3} - 560.3333 = 1.0000$

SS.Treatment $= \dfrac{E(Ex_i)^2}{ni} - CT$

$= \dfrac{(9 + 1521 + 1600)}{4} - 560.3333 = 222.1667$

Table 51 ANOVA table of survived mice during day 90 and 180.

Source	df	SS.	MS	F
Treatment	2	222.1667	111.0837	102.5417
Block	3	1.0000	0.3333	0.3077
Residual	6	6.5000	1.0833	
Total	11	229.6667		

Treatment ; from table, $F_{0.05}$ (2, 6) = 5.14

102.5417 > $F_{0.05}$; Reject the null hypothesis (H_{10}) (P>0.05)

Block ; from table, $F_{0.05}$ (3, 6) = 4.76

0.3077 < $F_{0.05}$; Accept the null hypothesis (H_{20}) (P<0.05)

Hence, there are significant difference in number of survived mice among tetanus toxoid preparation but there are no significant difference in number of survived mice at each time period.

Duncan's New Multiple Range Test for Treatment (Data from table 50)

$Sx = \sqrt{1.0833/4} = 0.5204$

df of error = 6

P value	2	3
SSR	3.46	3.58
LSR = (SSR) Sx	1.8006	1.8630

Preparation	TT	TTM	TT+TTM
X	0.75	9.75	10

TT + TTM, TT	= 10 - 0.75	=	9.25 > 1.8630	S
TT + TTM, TTM	= 10 - 9.75	=	0.25 < 1.8006	NS
TTM, TT	= 9.75 - 0.75	=	9.00 > 1.8006	S

b) Stability of potency testing

1) Result from table 11 were filled in contingency table 52, 54 and 56

Table 52 Contingency table of survived mice during day 0 and 15.

Day\Month	0	3	6	9	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
7	5	5	5	4	19	361	4.75
15	9	8	9	8	34	1156	8.50
Ex_i	14	13	14	12	53		
$(Ex_i)^2$	196	169	196	144			
$\overline{x_i}$	3.50	3.25	3.50	3.00			

Hypothesis

Treatment

H_{1O} : There are no significant difference in number of survived mice at each month storage period of same preparation.

H_{1A} : There are significant difference in number of survived mice at each month storage period of same preparation.

Block

H_{2O} : There are no significant difference in number of survived mice at each time period.

H_{2A} : There are significant difference in number of survived mice at each time period.

Calculation data from table 52

$Ex_{ij}^2 = 381$

$$\text{Correction Term (CT)} = \frac{(E_{ij}\, x_{ij})^2}{N} = \frac{(53)^2}{(4)(4)} = 175.5625$$

$$\text{SS.Total} = Ex_{ij}^2 - CT$$

$$= 381 - 175.5625$$

$$= 205.4375$$

$$\text{SS.Block} = \frac{E\,(Ex_j)^2}{nj} - CT$$

$$= \frac{(361 + 1156)}{4} - 175.5625$$

$$= 203.6875$$

$$\text{SS.Treatment} = \frac{E(Ex_i)^2}{ni} - CT$$

$$= \frac{(196 + 169 + 196 + 144)}{4} - 175.5625$$

$$= 0.6875$$

Table 53 ANOVA table of survived mice during day 0 and 15.

Source	df	SS.	MS	F
Treatment	3	0.6875	0.2292	1.9401
Block	3	203.6875	67.8958	574.9001
Residual	9	1.0625	0.1181	
Total	15	205.4375		

degree of freedom of total = (4)(4) - 1 = 15

degree of freedom of block = 4 - 1 = 3

degree of freedom of treatment = 4 - 1 = 3

degree of freedom of residual = (4 - 1) (4 - 1) = 9

Treatment ; from table, $F_{0.05}$ (3, 9) = 3.86

1.9401 < $F_{0.05}$; Accept the null hypothesis (H_{10}) (P<0.05)

Block ; from table, $F_{0.05}$ (3, 9) = 3.86

574.9001 > $F_{0.05}$; Reject the null hypothesis (H_{20}) (P>0.05)

Hence, there are no significant difference in number of survived mice at each month period but there are significant difference in number of survived mice at each time period.

Duncan's New Multiple Range Test for block (Data from table 52)

$Sx = \sqrt{0.1181/4} = 0.1718$

df of error = 9

P value	2	3	4
SSR	3.20	3.34	3.41
LSR = (SSR) Sx	0.5498	0.5738	0.5858

Day	0	3	7	15
X	0	0	4.75	8.50

Day	15, 0	=	8.50 - 0	=	8.50 > 0.5858	S
Day	15, 3	=	8.50 - 0	=	8.50 > 0.5738	S
Day	15, 7	=	8.50 - 4.75	=	3.75 > 0.5498	S
Day	7, 0	=	4.75- 0	=	4.75 > 0.5738	S
Day	7, 3	=	4.75 - 0	=	4.75 > 0.5498	S
Day	3, 0	=	0 - 0	=	0 < 0.5498	NS

Table 54 Contingency table of survived mice during day 30 and 75.

Day\Month	0	3	6	9	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
30	10	10	10	10	40	1600	10
45	10	10	10	10	40	1600	10
60	10	10	9	9	38	1444	9.50
75	8	7	6	5	26	676	6.50
Ex_i	38	37	35	34	144		
$(Ex_i)^2$	1444	1369	1225	1156			
$\overline{x_i}$	9.50	9.25	8.75	8.50			

Data from table 54

$Ex_{ij}^2 = 1336$

$$CT = \frac{(E_{ij}\, x_{ij})^2}{N} = \frac{(144)^2}{(4)(4)} = 1296.00$$

$$SS.Total = Ex_{ij}^2 - CT$$

$$= 1336 - 1296$$

$$= 40$$

$$SS.Block = \frac{E\,(Ex_j)^2}{nj} - CT$$

$$= \frac{(1600 + 1600 + 1444 + 676)}{4} - 1296 = 34$$

$$SS.Treatment = \frac{E(Ex_i)^2}{ni} - CT$$

$$= \frac{(1444 + 1369 + 1225 + 1156)}{4} - 1296 = 2.5$$

Table 55 ANOVA table of survived mice during day 30 and 75.

Source	df	SS.	MS	F
Treatment	3	2.5	0.8333	2.1427
Block	3	34	11.3333	29.1419
Residual	9	3.5	0.3889	
Total	15	40		

Treatment ; from table, $F_{0.05}$ (3, 9) = 3.86

2.1427 < $F_{0.05}$; Accept the null hypothesis (H_{10}) ($P<0.05$)

Block ; from table, $F_{0.05}$ (3, 9) = 3.86

29.1419 > $F_{0.05}$; Reject the null hypothesis (H_{20}) ($P>0.05$)

Hence, there are no significant difference in number of survived mice at each month period but there are significant difference in number of survived mice at each time period.

Duncan's New Multiple Range Test for block (Data from table 54)

$Sx = \sqrt{0.3889/4} = 0.3118$

df of error = 9

P value	2	3	4
SSR	3.20	3.34	3.41
LSR = (SSR) Sx	0.9978	1.0414	1.0632

Day	75	60	45	30
X	6.50	9.50	10.00	10.00

Day 30, 75 = 10 - 6.50 = 3.5 > 1.0632 S

Day 30, 60 = 10 - 9.50 = 0.5 < 1.0414 NS

Day 30, 45 = 10 - 10 = 0 < 0.9978 NS

Day 45, 75 = 10 - 6.50 = 3.5 > 1.0414 S

Day 45, 60 = 10 - 9.50 = 0.5 < 0.9978 NS

Day 60, 75 = 9.50 - 6.50 = 3.0 > 0.9978 S

Table 56 Contingency table of survived mice during day 90 and 180.

Day\Month	0	3	6	9	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
90	5	5	4	3	17	289	4.25
120	0	0	0	0	0	0	0
150	0	0	0	0	0	0	0
180	0	0	0	0	0	0	0
Ex_i	5	5	4	3	17		
$(Ex_i)^2$	25	25	16	9			
$\overline{x_i}$	1.25	1.25	1.0	0.75			

Data from table 56

$Ex_{ij}^2 \quad = \quad 75$

$CT \quad = \quad \frac{(E_{ij}\, x_{ij})^2}{N} = \frac{(17)^2}{(4)(4)} = 18.0625$

$SS.Total \quad = \quad Ex_{ij}^2 - CT$

$= \quad 75 - 18.0625$

$= \quad 56.9375$

$SS.Block \quad = \quad \frac{E\,(Ex_j)^2}{nj} - CT$

$= \quad \frac{(289 + 0 + 0 + 0)}{4} - 18.0625 = 54.1875$

$SS.Treatment \quad = \quad \frac{E(Ex_i)^2}{ni} - CT$

$= \quad \frac{(25 + 25 + 16 + 6)}{4} - 18.0625 = 0.6875$

Table 57 ANOVA table of survived mice during day 90 and 180.

Source	df	SS.	MS	F
Treatment	3	0.6875	0.2292	1.00
Block	3	54.1875	180625	78.81
Residual	9	2.0625	0.2292	
Total	15	56.9375		

Treatment ; from table, $F_{0.05}$ (3, 9) = 3.86

1.00 < $F_{0.05}$; Accept the null hypothesis (H_{10}) (P<0.05)

Block ; from table, $F_{0.05}$ (3, 9) = 3.86

78.81 > $F_{0.05}$; Reject the null hypothesis (H_{20}) (P>0.05)

Hence, there are no significant difference in number of survived mice at each month period but there are significant difference in number of survived mice at each time period.

Duncan's New Multiple Range Test for block (Data from table 56)

$Sx = \sqrt{0.2292/4} = 0.2394$

df of error = 9

P value	2	3	4
SSR	3.20	3.34	3.41
LSR = (SSR) Sx	0.7661	0.7996	0.8164

Day	180	150	120	90
X	0	0	0	4.25

Day	90, 180	=	4.25 - 0	=	4.25	> 0.8164	S
Day	90, 150	=	4.25 - 0	=	4.25	> 0.7996	S
Day	90, 120	=	4.25 - 0	=	4.25	> 0.7661	S
Day	120, 180	=	0 - 0	=	0	< 0.7996	NS
Day	120, 150	=	0 - 0	=	0	< 0.7661	NS
Day	150, 180	=	0 - 0	=	0	< 0.7667	NS

2) Result from table 13 were filled in contingency table 58, 60 and 62

Table 58 Contingency table of survived mice during day 0 and 15.

Day\Month	0	3	6	9	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
7	0	1	0	2	3	9	0.75
15	5	6	7	6	24	576	6.00
Ex_i	5	7	7	8	27		
$(Ex_i)^2$	25	49	49	64			
$\overline{x_i}$	1.25	1.75	1.75	2			

Data from table 58

$$Ex_{ij}^2 = 151$$

$$CT = \frac{(E_{ij}\, x_{ij})^2}{N} = \frac{(27)^2}{(4)(4)} = 45.5625$$

$$SS.Total = Ex_{ij}^2 - CT$$

$$= 151 - 45.5625 = 105.4375$$

$$SS.Block = \frac{E\,(Ex_j)^2}{nj} - CT$$

$$= \frac{(0 + 0 + 9 + 576)}{4} - 45.5625 = 100.6875$$

$$SS.Treatment = \frac{E(Ex_i)^2}{ni} - CT$$

$$= \frac{(25 + 49 + 49 + 64)}{4} - 45.5625 = 1.1875$$

Table 59 ANOVA table of survived mice during day 0 and 15.

Source	df	SS.	MS	F
Treatment	3	1.1875	0.3958	1.0000
Block	3	100.6875	33.5625	84.7966
Residual	9	3.5625	0.3958	
Total	15	105.4375		

Treatment ; from table, $F_{0.05}$ (3, 9) = 3.86

1.0000 < $F_{0.05}$; Accept the null hypothesis (H_{10}) (P<0.05)

Block ; from table, $F_{0.05}$ (3, 9) = 3.86

84.7966 > $F_{0.05}$; Reject the null hypothesis (H_{20}) (P>0.05)

Hence, there are no significant difference in number of survived mice at each month period but there are significant difference in number of survived mice at each time period.

Duncan's New Multiple Range Test for block (Data from table 58)

$Sx = \sqrt{0.3958/4} = 0.3146$

df of error = 9

P value	2	3	4
SSR	3.20	3.34	3.41
LSR = (SSR) Sx	1.0067	1.0508	1.0728

Day	0	3	7	15
X	0	0	0.75	6.00

Day 15, 0 = 6.00 - 0 = 6.00 > 1.0728 S

Day 15, 3 = 6.00 - 0 = 6.00 > 1.0508 S

Day 15, 7 = 6.00 - 0.75 = 5.25 > 1.0067 S

Day 7, 0 = 0.75 - 0 = 0.75 < 1.0508 NS

Day 7, 3 = 0.75 - 0 = 0.75 < 1.0067 NS

Day 3, 0 = 0 - 0 = 0 < 1.0067 NS

Table 60 Contingency table of survived mice during day 30 and 75.

Day\Month	0	3	6	9	Ex_j	$(Ex_j)^2$	$\overline{x}_j$
30	9	10	10	9	38	1444	9.50
45	10	10	10	10	40	1600	10.00
60	10	10	10	10	40	1600	10.00
75	10	10	10	10	40	1600	10.00
Ex_i	39	40	40	39	158		
$(Ex_i)^2$	1521	1600	1600	1521			
$\overline{x}_i$	9.75	10.00	10.00	9.75			

Data from table 60

$$Ex_{ij}^2 = 1562$$

$$CT = \frac{(E_{ij}\,x_{ij})^2}{N} = \frac{(158)^2}{(4)(4)} = 1560.2500$$

$$SS.Total = Ex_{ij}^2 - CT$$

$$= 1562 - 1560.2500$$

$$= 1.7500$$

$$SS.Block = \frac{E\,(Ex_j)^2}{nj} - CT$$

$$= \frac{(1444 + 1600 + 1600 + 1600)}{4} - 1560.2500 = 0.7500$$

$$SS.Treatment = \frac{E(Ex_i)^2}{ni} - CT$$

$$= \frac{(1521 + 1600 + 1600 + 1521)}{4} - 156.2500 = 0.2500$$

Table 61 ANOVA table of survived mice during day 30 and 75.

Source	df	SS.	MS	F
Treatment	3	0.2500	0.0833	1.0000
Block	3	0.7500	0.2500	3.0012
Residual	9	0.7500	0.0833	
Total	15	1.7500		

Treatment ; from table, $F_{0.05}$ (3, 9) = 3.86

$1.0000 < F_{0.05}$; Accept the null hypothesis (H_{10}) (P<0.05)

Block ; from table, $F_{0.05}$ (3, 9) = 3.86

$3.0012 < F_{0.05}$; Accept the null hypothesis (H_{20}) (P<0.05)

Hence, there are no significant difference in number of survived mice both among each month period and each time period.

Table 62 Contingency table of survived mice during day 90 and 180.

Day\Month	0	3	6	9	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
90	10	10	10	9	39	1521	9.75
120	10	9	9	10	38	1444	9.50
150	10	10	10	10	40	1600	10
180	10	10	10	10	40	1600	10
Ex_i	40	39	39	39	157		
$(Ex_i)^2$	1600	1521	1521	1521			
$\overline{x_i}$	10	9.75	9.75	9.75			

Data from table 62

$$Ex_{ij}^2 = 1543$$

$$CT = \frac{(E_{ij}\,x_{ij})^2}{N} = \frac{(157)^2}{(4)(4)} = 1540.5625$$

$$SS.Total = Ex_{ij}^2 - CT$$

$$= 1543 - 1540.5625 = 2.4375$$

$$SS.Block = \frac{E\,(Ex_j)^2}{nj} - CT$$

$$= \frac{(1521 + 1444 + 1600 + 1600)}{4} - 1540.5625$$

$$= 0.6875$$

$$SS.Treatment = \frac{E(Ex_i)^2}{ni} - CT$$

$$= \frac{(1600 + 1521 + 1521 + 1521)}{4} - 1540.5625 = 0.1875$$

Table 63 ANOVA table of survived mice during day 90 and 180.

Source	df	SS.	MS	F
Treatment	3	0.1875	0.0625	0.3600
Block	3	0.6875	0.2292	1.3203
Residual	9	1.5625	0.1736	
Total	15	2.4375		

Treatment ; from table, $F_{0.05}$ (3, 9) = 3.86

0.3600 < $F_{0.05}$; Accept the null hypothesis (H_{10}) (P<0.05)

Block ; from table, $F_{0.05}$ (3, 9) = 3.86

1.3203 < $F_{0.05}$; Accept the null hypothesis (H_{20}) (P<0.05)

Hence, there are no significant difference in number of survived mice both among each month period and each time period.

3) Result from table 15 were filled in contingency table 64, 66 and 68

Table 64 Contingency table of survived mice during day 0 and 15.

Day\Month	0	3	6	9	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
7	5	4	6	5	20	400	5.00
15	8	8	9	8	33	1089	8.25
Ex_i	13	12	15	13	53		
$(Ex_i)^2$	169	144	225	169			
$\overline{x_i}$	3.25	3.00	3.75	3.25			

Calculation data from table 64

$Ex_{ij}^2 = 375$

$CT = \frac{(E_{ij}\, x_{ij})^2}{N} = \frac{(53)^2}{(4)(4)} = 175.5625$

$SS.Total = Ex_{ij}^2 - CT$

$= 375 - 175.5625 = 199.4375$

$SS.Block = \frac{E\,(Ex_j)^2}{nj} - CT$

$= \frac{(0 + 0 + 400 + 1089)}{4} - 175.5625 = 196.6875$

$SS.Treatment = \frac{E(Ex_i)^2}{ni} - CT$

$= \frac{(169 + 144 + 225 + 169)}{4} - 175.5625 = 1.1875$

Table 65 ANOVA table of survived mice during day 0 and 15.

Source	df	SS.	MS	F
Treatment	3	1.1875	0.3958	2.2799
Block	3	196.6875	65.5625	377.6642
Residual	9	1.5625	0.1736	
Total	15	199.4375		

Treatment ; from table, $F_{0.05}$ (3, 9) = 3.86

2.2799 < $F_{0.05}$; Accept the null hypothesis (H_{10}) (P<0.05)

Block ; from table, $F_{0.05}$ (3, 9) = 3.86

377.6642 > $F_{0.05}$; Reject the null hypothesis (H_{20}) (P>0.05)

Hence, there are no significant difference in number of survived mice at each month period but there are significant difference in number of survived mice at each time period.

Duncan's New Multiple Range Test for block (Data from table 64)

$Sx = \sqrt{0.1736/4} = 0.2083$

df of error = 9

P value	2	3	4
SSR	3.20	3.34	3.41
LSR = (SSR) Sx	0.6666	0.6957	0.7103

Day	0	3	7	15
X	0	0	5.00	8.25

Day	15, 0	=	8.25 - 0	=	8.25	> 0.7103	S
Day	15, 3	=	8.25 - 0	=	8.25	> 0.6957	S
Day	15, 7	=	8.25 - 5.00	=	3.25	> 0.6666	S
Day	7, 0	=	5.00 - 0	=	5.00	> 0.6957	S
Day	7, 3	=	5.00 - 0	=	5.00	> 0.6666	S
Day	3, 0	=	0 - 0	=	0	< 0.6666	NS

Table 66 Contingency table of survived mice during day 30 and 75.

Day\Month	0	3	6	9	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
30	10	10	10	10	40	1600	10
45	10	10	10	10	40	1600	10
60	10	10	10	10	40	1600	10
75	10	10	10	10	40	1600	10
Ex_i	40	40	40	40	160		
$(Ex_i)^2$	1600	1600	1600	1600			
$\overline{x_i}$	10	10	10	10			

Data from table 66

$Ex_{ij}^2 = 1600$

$$CT = \frac{(E_{ij}\, x_{ij})^2}{N} = \frac{(160)^2}{(4)(4)} = 1600$$

$$SS.Total = Ex_{ij}^2 - CT$$

$$= 1600 - 1600 = 0$$

$$SS.Block = \frac{E\,(Ex_j)^2}{nj} - CT$$

$$= \frac{(1600 + 1600 + 1600 + 1600)}{4} - 1600$$

$$= 0$$

$$SS.Treatment = \frac{E(Ex_i)^2}{ni} - CT$$

$$= \frac{(1600 + 1600 + 1600 + 1600)}{4} - 1600 = 0$$

Table 67 ANOVA table of survived mice during day 30 and 75.

Source	df	SS.	MS	F
Treatment	3	0	0	-
Block	3	0	0	-
Residual	9	0	0	-
Total	15	0		

Treatment ; from table, $F_{0.05}$ (3, 9) = 3.86

Accept the null hypothesis (H_{10}) (P<0.05)

Block ; from table, $F_{0.05}$ (3, 9) = 3.86

Accept the null hypothesis (H_{20}) (P<0.05)

Hence, there are no significant difference in number of survived mice both among each month period and each time period.

Table 68 Contingency table of survived mice during day 90 and 180.

Day\Month	0	3	6	9	Ex_j	$(Ex_j)^2$	$\overline{x}_j$
90	10	10	10	10	40	1600	10
120	10	10	10	10	40	1600	10
150	10	10	9	10	39	1521	9.75
180	10	10	10	10	40	1600	10
Ex_i	40	40	39	40	159		
$(Ex_i)^2$	1600	1600	1521	1600			
$\overline{x}_i$	10	10	9.75	10			

Data from table 68

$Ex_{ij}^2 = 1581$

$$CT = \frac{(E_{ij}\, x_{ij})^2}{N} = \frac{(159)^2}{(4)(4)} = 1580.0625$$

$$SS.Total = Ex_{ij}^2 - CT$$

$$= 1581 - 1580.0625 = 0.9375$$

$$SS.Block = \frac{E\ (Ex_j)^2}{nj} - CT$$

$$= \frac{(1600 + 1600 + 1521 + 1600)}{4} - 1580.0625$$

$$= 0.1875$$

$$SS.Treatment = \frac{E(Ex_i)^2}{ni} - CT$$

$$= \frac{(1600 + 1600 + 1521 + 1600)}{4} - 1580.0625 = 0.1875$$

Table 69 ANOVA table of survived mice during day 90 and 180.

Source	df	SS.	MS	F
Treatment	3	0.1875	0.0625	1.0000
Block	3	0.1875	0.0625	1.0000
Residual	9	0.5625	0.0625	
Total	15	0.9375		

Treatment ; from table, $F_{0.05}$ (3, 9) = 3.86

1.0000 < $F_{0.05}$; Accept the null hypothesis (H_{10}) (P<0.05)

Block ; from table, $F_{0.05}$ (3, 9) = 3.86

1.0000 < $F_{0.05}$; Accept the null hypothesis (H_{20}) (P<0.05)

Hence, there are no significant difference in number of survived mice both among each month period and each time period.

C) Antibody titers determination

1) Results from table 17 were filled in contingency table 70, 72 and 74

Table 70 Contingency table of antibody titers of mouse anti - tetanus serum during day 0 and 15.

Day\Preparation	TT	TTM	TT+TTM	Ex_j	$(Ex_j)^2$	$\overline{x}_j$
0	10	10	10	30	900	10.00
3	10	10	10	30	900	10.00
7	50	10	50	110	12100	36.37
15	250	250	250	750	562500	250
Ex_i	320	280	320	920		
$(Ex_i)^2$	102400	78400	102400			
$\overline{x}_i$	80	70	80			

Hypothesis

Treatment

H_{1O} : There are no significant difference in titer level among tetanus toxoid preparation.

H_{1A} : There are significant difference in titer level among tetanus toxoid preparation.

Block

H_{2O} : There are no significant difference in titer level at each time period.

H_{2A} : There are significant difference in titer level at each time period.

Calculation dat from table 70

$Ex_{ij}^2 \quad = \quad 193200$

$CT \quad = \quad \frac{(E_{ij}\ x_{ij})^2}{N} = \frac{(920)^2}{(4)(3)} = 70533.33$

$SS.Total \quad = \quad Ex_{ij}^2 - CT$

$= 193200 - 70533.33$

$= 122666.67$

$SS.Block \quad = \quad \frac{E\ (Ex_j)^2}{nj} - CT$

$= \frac{(900 + 900 + 12100 + 562500)}{3} - 70533.33$

$= 121600.00$

$SS.Treatment \quad = \quad \frac{E(Ex_i)^2}{ni} - CT$

$= \frac{(102400 + 78400 + 102400)}{4} - 70533.33$

$= 266.67$

Table 71 ANOVA table of antibody titers of mouse anti - tetanus serum during day 0 and 15.

Source	df	SS.	MS	F
Treatment	2	266.67	133.34	1.0000
Block	3	121600	40533.33	304.01
Residual	6	800	133.33	
Total	11	122666.67		

Treatment ; from table, $F_{0.05}$ (2, 6) = 5.14

1.0000 < $F_{0.05}$; Accept the null hypothesis (H_{10}) (P<0.05)

Block ; from table, $F_{0.05}$ (3, 6) = 4.76

304.01 > $F_{0.05}$; Reject the null hypothesis (H_{20}) (P>0.05)

Hence, there are no significant difference in titer level among tetanus toxoid preparation but there are significant difference in titer level at each time period.

Duncan's New Multiple Range Test for block (Data from table 70)

Sx = $\sqrt{3022.22/3}$ = 31.74

df of error = 6

P value	2	3	4
SSR	3.46	3.58	3.64
LSR = (SSR) Sx	109.8204	113.6292	115.5336

Day	0	3	7	15
X	10.00	10.00	36.67	183.33

Day 15, 0 = 183.33 - 10.00 = 173.33 > 115.5336 S

Day 15, 3 = 183.33 - 0 = 173.33 > 133.6292 S

Day 15, 7 = 183.33 - 3.667 = 146.66 > 109.8204 S

Day 7, 0 = 36.67 - 10.00 = 26.67 < 113.6292 NS

Day 7, 3 = 36.67 - 10.00 = 26.67 < 109.8204 NS

Day 3, 0 = 10 - 10 = 0 < 109.8204 NS

Table 72 Contingency table of antibody titers of mouse anti - tetanus serum during day 30 and 75.

Day	TT	TTM	TT+TTM	Σx_j	$(\Sigma x_j)^2$	$\overline{x_j}$
30	250	1250	1250	2750	7562500	916.67
45	1250	1250	1250	3750	14062500	1250.00
60	1250	1250	6250	8750	76562500	2916.67
75	250	6250	1250	7750	60062500	2583.33
Σx_i	3000	10000	10000	23000		
$(\Sigma x_i)^2$	9000000	100000000	100000000			
$\overline{x_i}$	750	2500	2500			

Data from table 72

Ex_{ij}^{2} = 90750000

CT $= \frac{(E_{ij}\, x_{ij})^2}{N} = \frac{(23000)^2}{(4)(3)} = 44083333.33$

SS.Total $= Ex_{ij}^{2} - CT$

$= 90750000 - 44083333.33$

$= 46666666.67$

SS.Block $= \frac{E\,(Ex_{j})^2}{nj} - CT$

$= \frac{(7562500 + 14062500 + 76562500 + 60062500)}{3} - 44083333.33$

$= 8666666.67$

SS.Treatment $= \frac{E(Ex_{i})^2}{ni} - CT$

$= \frac{(9000000 + 100000000 + 100000000)}{4} - 44083333.33$

$= 8166666.67$

<u>Table 73</u> ANOVA table of antibody titers of mouse anti - tetanus serum during day 30 and 75.

Source	df	SS.	MS	F
Treatment	2	8166666.67	4083333.34	0.8212
Block	3	8666666.67	2888888.89	0.5810
Residual	6	29833333.33	4972222.22	
Total	11	46666666.67		

Treatment ; from table, $F_{0.05}$ (2, 6) = 5.14

$0.8212 < F_{0.05}$; Accept the null hypothesis (H_{10}) ($P<0.05$)

Block ; from table, $F_{0.05}$ (3, 6) = 4.76

$0.5810 < F_{0.05}$; Accept the null hypothesis (H_{20}) ($P<0.05$)

Hence, there are no significant difference in titer level both among tetanus toxoid preparation and each time period.

Table 74 Contingency table of antibody titers of mouse anti - tetanus serum during day 90 and 180.

Day	TT	TTM	TT+TTM	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
90	250	1250	1250	2750	7562500	916.67
120	10	1250	1250	2510	6300100	836.67
150	10	1250	1250	2510	6300100	836.67
180	10	1250	1250	2510	6300100	836.67
Ex_i	280	5000	5000	10280		
$(Ex_i)^2$	78400	25000000	25000000			
$\overline{x_i}$	70	1250	1250			

Data from table 74

$Ex_{ij}^2 = 12562800$

$$CT = \frac{(E_{ij}\,x_{ij})^2}{N} = \frac{(10280)^2}{(4)(3)} = 8806533.3330$$

$$SS.Total = Ex_{ij}^2 - CT$$

$$= 12562800 - 8806533.3330$$

$$= 3756266.6670$$

$$SS.Block = \frac{E\,(Ex_j)^2}{nj} - CT$$

$$= \frac{(7562500 + 6300100 + 6300100 + 6300100)}{3} - 8806533.3330$$

$$= 14400.0030$$

$$SS.Treatment = \frac{E(Ex_i)^2}{ni} - CT$$

$$= \frac{(78400 + 25000000 + 25000000)}{4} - 8806533.3330$$

$$= 3713066.667$$

Table 75 ANOVA table of antibody titers of mouse anti - tetanus serum during day 90 and 180.

Source	df	SS.	MS	F
Treatment	2	3713066.667	1856533.334	3867778
Block	3	14400.003	4800.001	1.0000
Residual	6	28799.997	4799.999	
Total	11	3756266.667		

Treatment ; from table, $F_{0.05}$ (2, 6) = 5.14

386.7778 > $F_{0.05}$; Reject the null hypothesis (H_{10}) (P>0.05)

Block ; from table, $F_{0.05}$ (3, 6) = 4.76

1.0000 < $F_{0.05}$; Accept the null hypothesis (H_{20}) (P<0.05)

Hence, there are significant difference in titer level among tetanus toxoid preparation but there are no significant difference in titer level at each time period.

Duncan's New Multiple Range Test for Treatment (Data from table 74)

$$Sx = \sqrt{4799.999/4} = 34.64$$

df of error = 6

P value	2	3
SSR	3.46	3.58
LSR = (SSR) Sx	119.8544	124.0112

Preparation	TT	TTM	TT+TTM
$\bar{X}$	70	1250	1250

TT + TTM, TT = 1250 - 70 = 1180 > 124.0112 S

TT + TTM, TTM = 1250 - 1250 = 0 < 119.8544 NS

TTM, TT = 1250 - 70 = 1180 > 119.8544 S

2) Result from table 19 were filled in contingency 76, 78 and 80

Table 76 Contingency table of antibody titers of mouse anti - tetanus serum during day 0 and 15.

Day\Preparation	TT	TTM	TT+TTM	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
0	10	10	10	30	900	10
3	10	10	10	30	900	10
7	50	10	50	110	12100	36.67
15	250	250	250	750	562500	250
Ex_i	320	280	320	920		
$(Ex_i)^2$	102400	78400	102400			
$\overline{x_i}$	80	70	80			

Calculation data from table 76

$Ex_{ij}^2 = 193200$

$$CT = \frac{(E_{ij}\, x_{ij})^2}{N} = \frac{(920)^2}{(4)(3)} = 70533.33$$

$$SS.Total = Ex_{ij}^2 - CT$$

$$= 193200 - 70533.33$$

$$= 122666.67$$

$$SS.Block = \frac{E\ (Ex_j)^2}{nj} - CT$$

$$= \frac{(900 + 900 + 12100 + 562500)}{3} - 70533.33$$

$$= 121600.00$$

$$SS.Treatment = \frac{E(Ex_i)^2}{ni} - CT$$

$$= \frac{(102400 + 78400 + 102400)}{4} - 70533.33$$

$$= 266.67$$

<u>Table 77</u> ANOVA table of antibody titers of mouse anti - tetanus serum during day 0 and 15.

Source	df	SS.	MS	F
Treatment	2	266.67	133.34	1.0000
Block	3	121600	40533.33	304.01
Residual	6	800	133.33	
Total	11	122666.67		

Treatment ; from table, $F_{0.05}$ (2, 6) = 5.14

1.0000 < $F_{0.05}$; Accept the null hypothesis (H_{10}) (P<0.05)

Block ; from table, $F_{0.05}$ (3, 6) = 4.76

304.01 > $F_{0.05}$; Reject the null hypothesis (H_{20}) (P>0.05)

Hence, there are no significant difference in titer level among tetanus toxoid preparation but there are significant difference in titer level at each time period.

Duncan's New Multiple Range Test for block (Data from table 76)

$Sx = \sqrt{133.33/3} = 6.67$

df of error = 6

P value	2	3	4
SSR	3.46	3.58	3.64
LSR = (SSR) Sx	23.0782	23.8786	24.2788

Day	0	3	7	15
X	10	10	36.67	250

Day 15, 0 = 250 - 10 = 240 > 24.2788 S

Day 15, 3 = 250 - 10 = 240 > 23.8786 S

Day 15, 7 = 250 - 36.67 = 213.33 > 23.0782 S

Day 7, 0 = 36.67 - 10 = 26.67 > 23.8786 S

Day 7, 3 = 36.67 - 10 = 26.67 > 23.0782 S

Day 3, 0 = 10 - 10 = 0 < 23.0782 NS

Table 78 Contingency table of antibody titers of mouse anti - tetanus serum during day 30 and 75.

Day\Preparation	TT	TTM	TT+TTM	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
30	250	1250	1250	2750	7562500	916.67
45	1250	1250	1250	3750	14062500	1250.00
60	1250	1250	6250	8750	76562500	2916.67
75	250	6250	1250	7750	60062500	2583.33
Ex_i	3000	10000	10000	23000		
$(Ex_i)^2$	9000000	100000000	100000000			
$\overline{x_i}$	750	2500	2500			

Data from table 78

$$Ex_{ij}^2 = 90750000$$

$$CT = \frac{(E_{ij}\ x_{ij})^2}{N} = \frac{(23000)^2}{(4)(3)} = 44083333.33$$

$$SS.Total = Ex_{ij}^2 - CT$$

$$= 90750000 - 44083333.33$$

$$= 46666666.67$$

$$SS.Block = \frac{E\ (Ex_j)^2}{nj} - CT$$

$$= \frac{(7562500 + 14062500 + 76562500 + 60062500)}{3} - 44083333.33$$

$$= 8666666.67$$

$$SS.Treatment = \frac{E(Ex_i)^2}{ni} - CT$$

$$= \frac{(9000000 + 100000000 + 100000000)}{4} - 44083333.33$$

$$= 8166666.67$$

Table 79 ANOVA table of antibody titers of mouse anti - tetanus serum during day 30 and 75.

Source	df	SS.	MS	F
Treatment	2	8166666.67	4083333.34	0.8212
Block	3	8666666.67	2888888.89	0.5810
Residual	6	29833333.33	4972222.22	
Total	11	46666666.67		

Treatment ; from table, $F_{0.05}$ (2, 6) = 5.14

$0.8212 < F_{0.05}$; Accept the null hypothesis (H_{10}) (P<0.05)

Block ; from table, $F_{0.05}$ (3, 6) = 4.76

$0.5810 < F_{0.05}$; Accept the null hypothesis (H_{20}) (P<0.05)

Hence, there are no significant difference in titer level both among tetanus toxoid preparation and each time period.

Table 80 Contingency table of antibody titers of mouse anti - tetanus serum during day 90 and 180.

Day\Preparation	TT	TTM	TT+TTM	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
90	250	1250	1250	2750	7562500	916.67
120	10	1250	1250	2510	6300100	836.67
150	10	1250	1250	2510	6300100	836.67
180	10	1250	1250	2510	6300100	836.67
Ex_i	280	5000	5000	10280		
$(Ex_i)^2$	78400	25000000	25000000			
$\overline{x_i}$	70	1250	1250			

Data from table 80

Σx_{ij}^2 = 12562800

CT $= \frac{(\Sigma_{ij} x_{ij})^2}{N} = \frac{(10280)^2}{(4)(3)} = 8806533.3330$

SS.Total $= \Sigma x_{ij}^2 - CT$

$= 12562800 - 8806533.3330$

$= 3756266.6670$

SS.Block $= \frac{\Sigma (\Sigma x_j)^2}{nj} - CT$

$= \frac{(7562500 + 6300100 + 6300100 + 6300100)}{3} - 8806533.3330$

$= 14400.0000$

SS.Treatment $= \frac{\Sigma(\Sigma x_i)^2}{ni} - CT$

$= \frac{(78400 + 25000000 + 25000000)}{4} - 8806533.3330$

$= 31713066.667$

Table 81 ANOVA table of antibody titers of mouse anti - tetanus serum during day 90 and 180.

Source	df	SS.	MS	F
Treatment	2	3713066.667	1856533.334	386.7778
Block	3	14400.0000	4800.0000	1.0000
Residual	6	28800	4800	
Total	11	3756266.667		

Treatment ; from table, $F_{0.05}$ (2, 6) = 5.14

386.7778 > $F_{0.05}$; Reject the null hypothesis (H_{10}) (P>0.05)

Block ; from table, $F_{0.05}$ (3, 6) = 4.76

1.0000 < $F_{0.05}$; Accept the null hypothesis (H_{20}) (P<0.05)

Hence, there are significant difference in titer level among tetanus toxoid preparation but there are no significant difference in titer level at each time period.

Duncan's New Multiple Range Test for Treatment (Data from table 80)

$S\bar{x} = \sqrt{4800/4} = 34.64$

df of error = 6

P value	2	3
SSR	3.46	3.58
LSR = (SSR) Sx	119.8544	124.0112

Preparation	TT	TTM	TT+TTM
$\bar{X}$	70	1250	1250

TT + TTM, TT = 1250 - 70 = 1180 > 124.0112 S

TT + TTM, TTM = 1250 - 1250 = 0 < 119.8544 NS

TTM, TT = 1250 - 70 = 1180 > 119.8544 S

3) Resolt from table 21 were filled in contingency table 82, 84 and 86

Table 82 Contingency table of antibody titers of mouse anti - tetanus serum during day 0 and 15.

Day\Preparation	TT	TTM	TT+TTM	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
0	10	10	10	30	900	10.00
3	10	10	10	30	900	10.00
7	50	10	50	110	12100	36.67
15	250	250	250	750	562500	250
Ex_i	320	280	320	920		
$(Ex_i)^2$	102400	78400	102400			
$\overline{x_i}$	80	70	80			

Calculation data from table 82

$Ex_{ij}^2 \quad = \quad 193200$

$$CT = \frac{(E_{ij}\ x_{ij})^2}{N} = \frac{(920)^2}{(4)(3)} = 70533.33$$

$$SS.Total = Ex_{ij}^2 - CT$$

$$= 193200 - 70533.33$$

$$= 122666.67$$

$$SS.Block = \frac{E\ (Ex_j)^2}{nj} - CT$$

$$= \frac{(900 + 900 + 12100 + 562500)}{3} - 70533.33$$

$$= 121600.00$$

$$SS.Treatment = \frac{E(Ex_i)^2}{ni} - CT$$

$$= \frac{(102400 + 78400 + 102400)}{3} - 70533.33$$

$$= 266.67$$

Table 83 ANOVA table of antibody titers of mouse anti - tetanus serum during day 0 and 15.

Source	df	SS.	MS	F
Treatment	2	266.67	133.34	1.0000
Block	3	121600	40533.33	304.01
Residual	6	800	133.33	
Total	11	122666.67		

Treatment ; from table, $F_{0.05}$ (2, 6) = 5.14

$1.0000 < F_{0.05}$; Accept the null hypothesis (H_{10})

Block ; from table, $F_{0.05}$ (3, 6) = 4.76

$304.01 > F_{0.05}$; Reject the null hypothesis (H_{20})

Hence, there are no significant difference in titer level among tetanus toxoid preparation but there are significant difference in titer level at each time period.

Duncan's New Multiple Range Test for block (Data from table 82)

$S\bar{x} = \sqrt{133.33/3} = 6.67$

df of error = 6

P value	2	3	4
SSR	3.46	3.58	3.64
LSR = (SSR) Sx	23.0782	23.8786	24.2788

Day	0	3	7	15
X	10.00	10.00	36.67	250

Day 15, 0 = 250 - 10 = 240 > 24.2788 S

Day 15, 3 = 250 - 10 = 240 > 23.8786 S

Day 15, 7 = 250 - 36.67 = 213.33 > 23.0782 S

Day 7, 0 = 36.67 - 10 = 26.67 > 23.8786 S

Day 7, 3 = 36.67 - 10 = 26.67 > 23.0782 S

Day 3, 0 = 10 - 10 = 0 < 23.0782 NS

Table 84 Contingency table of antibody titers of mouse anti - tetanus serum during day 30 and 75.

Day\Preparation	TT	TTM	TT+TTM	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
30	250	1250	1250	2750	7562500	916.67
45	1250	1250	1250	3750	14062500	1250.00
60	1250	1250	6250	8750	76562500	2916.67
75	250	6250	1250	7750	60062500	2583.33
Ex_i	3000	10000	10000	23000		
$(Ex_i)^2$	9000000	100000000	100000000			
$\overline{x_i}$	750	2500	2500			

Data from table 84

$$Ex_{ij}^{2} = 90750000$$

$$CT = \frac{(E_{ij}\ x_{ij})^{2}}{N} = \frac{(23000)^{2}}{(4)(3)} = 44083333.33$$

$$SS.Total = Ex_{ij}^{2} - CT$$

$$= 90750000 - 44083333.33$$

$$= 46666666.67$$

$$SS.Block = \frac{E\ (Ex_{j})^{2}}{nj} - CT$$

$$= \frac{(7562500 + 14062500 + 76562500 + 60062500)}{3} - 44083333.33$$

$$= 8666666.67$$

$$SS.Treatment = \frac{E(Ex_{i})^{2}}{ni} - CT$$

$$= \frac{(9000000 + 100000000 + 100000000)}{4} - 44083333.33$$

$$= 8166666.67$$

Table 85 ANOVA table of antibody titers of mouse anti - tetanus serum during day 30 and 75.

Source	df	SS.	MS	F
Treatment	2	8166666.67	4083333.34	0.8212
Block	3	8666666.67	2888888.89	0.5810
Residual	6	29833333.33	4972222.22	
Total	11	46666666.67		

Treatment ; from table, $F_{0.05}$ (2, 6) = 5.14

$0.8212 < F_{0.05}$; Accept the null hypothesis (H_{10}) (P<0.05)

Block ; from table, $F_{0.05}$ (3, 6) = 4.76

$0.5810 < F_{0.05}$; Accept the null hypothesis (H_{20}) (P<0.05)

Hence, there are no significant difference in titer level both among tetanus toxoid preparation and each time period.

Table 86 Contingency table of antibody titers of mouse anti - tetanus serum during day 90 and 180.

Day\Preparation	TT	TTM	TT+TTM	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
90	250	1250	1250	2750	7562500	916.67
120	10	1250	1250	2510	6300100	836.67
150	10	1250	1250	2510	6300100	836.67
180	10	1250	1250	2510	6300100	836.67
Ex_i	280	5000	5000	10280		
$(Ex_i)^2$	78400	25000000	25000000			
$\overline{x_i}$	70	1250	1250			

Data from table 86

$Ex_{ij}^2 = 12562800$

$$CT = \frac{(E_{ij}\ x_{ij})^2}{N} = \frac{(10280)^2}{(4)(3)} = 8806533.3330$$

$$SS.Total = Ex_{ij}^2 - CT$$

$$= 12562800 - 8806533.3330$$

$$= 3756266.6670$$

$$SS.Block = \frac{E\ (Ex_j)^2}{nj} - CT$$

$$= \frac{(7562500 + 6300100 + 6300100 + 6300100)}{3} - 8806533.3330$$

$$= 14400.0000$$

$$SS.Treatment = \frac{E(Ex_i)^2}{ni} - CT$$

$$= \frac{(78400 + 25000000 + 25000000)}{4} - 8806533.3330$$

$$= 3713066.6670$$

Table 87 ANOVA table of antibody titers of mouse anti - tetanus serum during day 90 and 180.

Source	df	SS.	MS	F
Treatment	2	3713066.667	1856533.334	386.7778
Block	3	14400.0000	4800.0000	1.0000
Residual	6	28800.0000	4800.0000	
Total	11	3756266.667		

Treatment ; from table, $F_{0.05}$ (2, 6) = 5.14

386.7778 > $F_{0.05}$; Reject the null hypothesis (H_{10}) (P>0.05)

Block ; from table, $F_{0.05}$ (3, 6) = 4.76

1.0000 < $F_{0.05}$; Accept the null hypothesis (H_{20}) (P<0.05)

Hence, there are significant difference in titer level among tetanus toxoid preparation but there are no significant difference in titer level at each time period.

Duncan's New Multiple Range Test for Treatment (Data from table 86)

$Sx = \sqrt{4800/4} = 34.64$

df of error = 6

P value	2	3
SSR	3.46	3.58
LSR = (SSR) Sx	119.8544	124.0112

Day	TT	TTM	TT+TTM
X	70	1250	1250

TT + TTM, TT = 1250 - 70 = 1180 > 124.0112 S

TT + TTM, TTM = 1250 - 1250 = 0 < 119.8544 NS

TTM, TT = 1250 - 70 = 1180 > 119.8544 S

4) Result from table 23 were filled in contingency table 88, 90 and 92

<u>Table 88</u> Contingency table of antibody titers of mouse anti - tetanus serum during day 0 and 15.

Day Preparation	TT	TTM	TT+TTM	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
0	10	10	10	30	900	10
3	10	10	10	30	900	10
7	50	10	50	110	12100	36.67
15	250	250	250	750	562500	
Ex_i	320	280	320	920		
$(Ex_i^2$	102400	78400	102400			
$\overline{x_i}$	80	70	80			

Data from table 88

$Ex_{ij}^{2} = 193200$

$CT = \frac{(E_{ij} x_{ij})^2}{N} = \frac{(920)^2}{(4)(3)} = 70533.33$

$SS.Total = Ex_{ij}^{2} - CT$

$= 193200 - 70533.33$

$= 122666.67$

$SS.Block = \frac{E\,(Ex_j)^2}{nj} - CT$

$= \frac{(900 + 900 + 12100 + 562500)}{3} - 70533.33$

$= 121600.00$

$SS.Treatment = \frac{E(Ex_i)^2}{ni} - CT$

$= \frac{(102400 + 78400 + 102400)}{4} - 70533.33$

$= 266.67$

Table 89 ANOVA table of antibody titers of mouse anti - tetanus serum during day 0 and 15.

Source	df	SS.	MS	F
Treatment	2	266.67	133.34	1.0000
Block	3	121600	40533.33	304.01
Residual	6	800	133.33	
Total	11	122666.67		

Treatment ; from table, $F_{0.05}$ (2, 6) = 5.14

1.00000 < $F_{0.05}$; Accept the null hypothesis (H_{10}) (P<0.05)

Block ; from table, $F_{0.05}$ (3, 6) = 4.76

304.01 > $F_{0.05}$; Reject the null hypothesis (H_{20}) (P>0.05)

Hence, there are no significant difference in titer level among tetanus toxoid preparation but there are significant difference in titer level at each time period.

Duncan's New Multiple Range Test for block (Data from table 88)

Sx = $\sqrt{133.33/3}$ = 6.67

df of error = 6

P value	2	3	4
SSR	3.46	3.58	3.64
LSR = (SSR) Sx	23.0782	23.8786	24.2788

Day	0	3	7	15
X	10	10	36.67	250

Day 15, 0 = 250 - 10 = 240 > 24.2788 S

Day 15, 3 = 250 - 10 = 240 > 23.8786 S

Day 15, 7 = 250 - 36.67 = 213.33 > 23.0782 S

Day 7, 0 = 36.67 - 10 = 26.67 > 23.8786 S

Day 7, 3 = 36.67 - 10 = 26.67 > 23.0782 S

Day 3, 0 = 10 - 10 = 0 < 23.0782 NS

Table 90 Contingency table of antibody titers of mouse anti - tetanus serum during day 30 and 75.

Day\Preparation	TT	TTM	TT+TTM	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
30	250	1250	1250	2750	7562500	916.67
45	1250	1250	1250	3750	14062500	1250.00
60	1250	1250	6250	8750	76562500	2916.67
75	250	6250	1250	7750	60062500	2583.33
Ex_i	3000	10000	10000	23000		
$(Ex_i)^2$	9000000	100000000	100000000			
$\overline{x_i}$	750	2500	2500			

Data from table 90

Ex_{ij}^2 = 90750000

CT $= \frac{(E_{ij}\ x_{ij})^2}{N} = \frac{(23000)^2}{(4)(3)} = 44083333.33$

SS.Total $= Ex_{ij}^2 - CT$

$= 90750000 - 44083333.33$

$= 46666666.67$

SS.Block $= \frac{E\ (Ex_j)^2}{nj} - CT$

$= \frac{(7562500 + 14062500 + 76562500 + 60062500)}{3} - 44083333.33$

$= 8666666.67$

SS.Treatment $= \frac{E(Ex_i)^2}{ni} - CT$

$= \frac{(9000000 + 100000000 + 100000000)}{4} - 44083333.33$

$= 8166666.67$

Table 91 ANOVA table of antibody titers of mouse anti - tetanus serum during day 30 and 75.

Source	df	SS.	MS	F
Treatment	2	8166666.67	4083333.34	0.8212
Block	3	8666666.67	2888888.89	0.5810
Residual	6	29833333.33	4972222.22	
Total	11	46666666.67		

Treatment ; from table, $F_{0.05}$ (2, 6) = 5.14

$0.8212 < F_{0.05}$; Accept the null hypothesis (H_{10}) ($P<0.05$)

Block ; from table, $F_{0.05}$ (3, 6) = 4.76

$0.5810 < F_{0.05}$; Accept the null hypothesis (H_{20}) ($P<0.05$)

Hence, there are no significant difference in titer level both among tetanus toxoid preparation and each time period.

Table 92 Contingency table of antibody titers of mouse anti - tetanus serum during day 90 and 180.

Day\Preparation	TT	TTM	TT+TTM	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
90	250	1250	1250	2750	7562500	916.67
120	10	1250	1250	2510	6300100	836.67
150	10	1250	1250	2510	6300100	836.67
180	10	1250	1250	2510	6300100	836.67
Ex_i	280	5000	5000	10280		
$(Ex_i)^2$	78400	25000000	25000000			
$\overline{x_i}$	70	1250	1250			

Data from table 92

$Ex_{ij}^2 \quad = \quad 12562800$

$$CT = \frac{(E_{ij}\,x_{ij})^2}{N} = \frac{(10280)^2}{(4)(3)} = 8806533.3330$$

$$SS.Total = Ex_{ij}^2 - CT$$

$$= 12562800 - 8806533.3330$$

$$= 3756266.6670$$

$$SS.Block = \frac{E\ (Ex_i)^2}{nj} - CT$$

$$= \frac{(7562500 + 6300100 + 6300100 + 6300100)}{3} - 8806533.3330$$

$$= 14400.0000$$

$$SS.Treatment = \frac{E(Ex_i)^2}{ni} - CT$$

$$= \frac{(78400 + 25000000 + 25000000)}{4} - 8806533.3330$$

$$= 3713066.667$$

Table 93 ANOVA table of antibody titers of mouse anti - tetanus serum during day 90 and 180.

Source	df	SS.	MS	F
Treatment	2	3713066.667	1856533.334	386.7778
Block	3	14400.0000	4800.0000	1.0000
Residual	6	28800	4800	
Total	11	3756266.667		

Treatment ; from table, $F_{0.05}$ (2, 6) = 5.14

386.7778 > $F_{0.05}$; Reject the null hypothesis (H_{10}) (P>0.05)

Block ; from table, $F_{0.05}$ (3, 6) = 4.76

1.0000 < $F_{0.05}$; Accept the null hypothesis (H_{20}) (P<0.05)

Hence, there are significant difference in titer level among tetanus toxoid preparation but there are no significant difference in titer level at each time period.

Duncan's New Multiple Range Test for Treatment (Data from table 92)

$Sx = \sqrt{4800/4} = 34.64$

df of error = 6

P value	2	3
SSR	3.46	3.58
LSR = (SSR) Sx	119.8544	124.0112

Day	TT	TTM	TT+TTM
X	70	1250	1250

TT + TTM, TT	= 1250 - 70	=	1180 > 124.0112	S
TT + TTM, TTM	= 1250 - 1250	=	0 < 119.8544	NS
TTM, TT	= 1250 - 70	=	1180 > 119.8544	S

d) Stability of tetanus toxoid preparation

1) Result from table 25 were filled in contingency table 94, 96 and 98

Table 94 Contingency table of antibody titers of mouse anti - tetanus serum during day 0 and 15.

Day\month	0	3	6	9	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
0	10	10	10	10	40	1600	10
3	10	10	10	10	40	1600	10
7	50	50	50	50	200	40000	50
15	250	250	250	250	1000	1000000	250
Ex_i	320	320	320	320	1280		
$(Ex_i)^2$	102400	102400	102400	102400			
$\overline{x_i}$	80	80	80	80			

Calculation data from table 94

$Ex_{ij}^2 = 260800$

$$CT = \frac{(E_{ij}\ x_{ij})^2}{N} = \frac{(1280)^2}{(4)(4)} = 102400$$

$$SS.Total = Ex_{ij}^2 - CT$$

$$= 260800 - 102400$$

$$= 158400$$

$$SS.Block = \frac{E\ (Ex_j)^2}{nj} - CT$$

$$= \frac{(1600 + 1600 + 40000 + 1000000)}{4} - 102400$$

$$= 158400$$

$$SS.Treatment = \frac{E(Ex_i)^2}{ni} - CT$$

$$= \frac{(102400 + 102400 + 102400 + 102400)}{4} - 102400$$

$$= 0$$

Table 95 ANOVA table of antibody titers of mouse anti - tetanus serum during day 0 and 15.

Source	df	SS.	MS	F
Treatment	3	0	0	-
Block	3	158400	52800	-
Residual	9	0	0	
Total	15	158400		

Treatment ; from table, $F_{0.05}$ (3, 9) = 3.86

$0 < F_{0.05}$; Accept the null hypothesis (H_{10}) (P<0.05)

Block ; from table, $F_{0.05}$ (3, 9) = 3.86

$0 < F_{0.05}$; Accept the null hypothesis (H_{20}) (P<0.05)

Hence, there are no significant difference in titer level both among each month period and each time period.

Table 96 Contingency table of antibody titers of mouse anti - tetanus serum during day 30 and 75.

Day\month	0	3	6	9	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
30	250	250	250	250	1000	1000000	250
45	1250	1250	1250	1250	5000	25000000	1250
60	1250	1250	1250	1250	5000	25000000	1250
75	250	250	250	250	1000	1000000	250
Ex_i	3000	3000	3000	3000	12000		
$(Ex_i)^2$	9000000	9000000	9000000	9000000			
$\overline{x_i}$	750	750	750	750			

Data from table 96

$$Ex_{ij}^2 = 13000000$$

$$CT = \frac{(E_{ij}\ x_{ij})^2}{N} = \frac{(12000)^2}{(4)(4)} = 9000000$$

$$SS.Total = Ex_{ij}^2 - CT$$

$$= 13000000 - 9000000$$

$$= 4000000$$

$$SS.Block = \frac{E\ (Ex_j)^2}{nj} - CT$$

$$= \frac{(1000000 + 25000000 + 25000000 + 1000000)}{4} - 9000000$$

$$= 4000000$$

$$SS.Treatment = \frac{E(Ex_i)^2}{ni} - CT$$

$$= \frac{(9000000 + 9000000 + 9000000 + 9000000)}{4} - 9000000$$

$$= 0$$

Table 97 ANOVA table of antibody titers of mouse anti - tetanus serum during day 30 and 75.

Source	df	SS.	MS	F
Treatment	3	0	0	-
Block	3	4000000	1333333.33	-
Residual	9	0	0	
Total	15	4000000		

Treatment ; from table, $F_{0.05}$ (3, 9) = 3.86

$0 < F_{0.05}$; Accept the null hypothesis (H_{10}) (P<0.05)

Block ; from table, $F_{0.05}$ (3, 9) = 3.86

$0 < F_{0.05}$; Accept the null hypothesis (H_{20}) (P<0.05)

Hence, there are no significant difference in titer level both among each month period and each time period.

Table 98 Contingency table of antibody titers of mouse anti - tetanus serum during day 90 and 180.

Day\month	0	3	6	9	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
90	250	250	250	250	1000	1000000	250
120	10	10	10	10	40	1600	10
150	10	10	10	10	40	1600	10
180	10	10	10	10	40	1600	10
Ex_i	280	280	280	280	1120		
$(Ex_i)^2$	78400	78400	78400	78400			
$\overline{x_i}$	70	70	70	70			

Data from table 98

$$Ex_{ij}^2 = 251200$$

$$CT = \frac{(E_{ij}\ x_{ij})^2}{N} = \frac{(1120)^2}{(4)(4)} = 78400$$

$$SS.Total = Ex_{ij}^2 - CT$$

$$= 251200 - 78400$$

$$= 172800$$

$$SS.Block = \frac{E\ (Ex_j)^2}{nj} - CT$$

$$= \frac{(1000000 + 1600 + 1600 + 1600)}{4} - 78400$$

$$= 172800$$

$$SS.Treatment = \frac{E(Ex_i)^2}{ni} - CT$$

$$= \frac{(78400 + 78400 + 78400 + 78400)}{4} - 78400$$

$$= 0$$

Table 99 ANOVA table of antibody titers of mouse anti - tetanus serum during day 90 and 180.

Source	df	SS.	MS	F
Treatment	3	0	0	-
Block	3	172800	57600	-
Residual	9	0	0	
Total	15	172800		

Treatment ; from table, $F_{0.05}$ (3, 9) = 3.86

$0 < F_{0.05}$; Accept the null hypothesis (H_{10}) ($P<0.05$)

Block ; from table, $F_{0.05}$ (3, 9) = 3.86

$0 < F_{0.05}$; Accept the null hypothesis (H_{20}) ($P<0.05$)

Hence, there are no significant difference in titer level both among each month period and each time period.

2) Result from table 26 were filled in contingency table 100, 102 and 104

Table 100 Contingency table of antibody titers of mouse anti - tetanus serum during day 0 and 15.

Day\month	0	3	6	9	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
0	10	10	10	10	40	1600	10
3	10	10	10	10	40	1600	10
7	10	10	10	10	40	1600	10
15	250	250	250	250	1000	1000000	250
Ex_i	280	280	280	280	1120		
$(Ex_i)^2$	78400	78400	78400	78400			
$\overline{x_i}$	70	70	70	70			

Data from table 100

Ex_{ij}^2 = 251200

CT $= \frac{(E_{ij} x_{ij})^2}{N} = \frac{(1120)^2}{(4)(4)} = 78400$

SS.Total $= Ex_{ij}^2 - CT$

$= 251200 - 78400$

$= 172800$

SS.Block $= \frac{E(Ex_j)^2}{nj} - CT$

$= \frac{(1600 + 1600 + 1600 + 1000000)}{4} - 78400$

$= 172800$

SS.Treatment $= \frac{E(Ex_i)^2}{ni} - CT$

$= \frac{(78400 + 78400 + 78400 + 78400)}{4} - 78400$

$= 0$

Table 101 ANOVA table of antibody titers of mouse anti - tetanus serum during day 0 and 15.

Source	df	SS.	MS	F
Treatment	3	0	0	-
Block	3	172800	57600	-
Residual	9	0	0	
Total	15	172800		

Treatment ; from table, $F_{0.05}$ (3, 9) = 3.86

$0 < F_{0.05}$; Accept the null hypothesis (H_{10}) (P<0.05)

Block ; from table, $F_{0.05}$ (3, 6) = 3.86

$0 < F_{0.05}$; Accept the null hypothesis (H_{20}) (P<0.05)

Hence, there are no significant difference in titer level both among each month period and each time period.

Table 102 Contingency table of antibody titers of mouse anti - tetanus serum during day 30 and 75.

Day\month	0	3	6	9	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
30	1250	1250	1250	1250	5000	25000000	1250
45	1250	1250	1250	1250	5000	25000000	1250
60	1250	1250	1250	1250	5000	25000000	1250
75	6250	6250	6250	6250	25000	625000000	6250
Ex_i	10000	10000	10000	10000	40000		
$(Ex_i)^2$	100000000	100000000	100000000	100000000			
$\overline{x_i}$	2500	2500	2500	2500			

Data from table 102

$$\Sigma x_{ij}^2 = 175000000$$

$$CT = \frac{(\Sigma_{ij} x_{ij})^2}{N} = \frac{(40000)^2}{(4)(4)} = 100000000$$

$$SS.Total = \Sigma x_{ij}^2 - CT$$

$$= 175000000 - 100000000$$

$$= 75000000$$

$$SS.Block = \frac{\Sigma (\Sigma x_{.j})^2}{nj} - CT$$

$$= \frac{(25000000 + 25000000 + 25000000 + 625000000)}{4} - 100000000$$

$$= 75000000$$

$$SS.Treatment = \frac{\Sigma(\Sigma x_{i.})^2}{ni} - CT$$

$$= \frac{(100000000 + 100000000 + 100000000 + 100000000)}{4} - 100000000$$

$$= 0$$

Table 103 ANOVA table of antibody titers of mouse anti - tetanus serum during day 30 and 75.

Source	df	SS.	MS	F
Treatment	3	0	0	-
Block	3	75000000	25000000	-
Residual	9	0	0	
Total	15	75000000		

Treatment ; from table, $F_{0.05}$ (3, 9) = 3.86

$0 < F_{0.05}$; Accept the null hypothesis (H_{10}) (P<0.05)

Block ; from table, $F_{0.05}$ (3, 9) = 3.86

$0 < F_{0.05}$; Accept the null hypothesis (H_{20}) (P<0.05)

Hence, there are no significant difference in titer level both among each month period and each time period.

Table 104 Contingency table of antibody titers of mouse anti - tetanus serum during day 90 and 180.

Day\month	0	3	6	9	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
90	1250	1250	1250	1250	5000	25000000	1250
120	1250	1250	1250	1250	5000	25000000	1250
150	1250	1250	1250	1250	5000	25000000	1250
180	1250	1250	1250	1250	5000	25000000	1250
Ex_i	5000	5000	5000	5000	20000		
$(Ex_i)^2$	25000000	25000000	25000000	25000000			
$\overline{x_i}$	1250	1250	1250	1250			

Data from table 104

$$Ex_{ij}^2 = 25000000$$

$$CT = \frac{(E_{ij}\, x_{ij})^2}{N} = \frac{(20000)^2}{(4)(4)} = 25000000$$

$$SS.Total = Ex_{ij}^2 - CT$$

$$= 25000000 - 25000000$$

$$= 0$$

$$SS.Block = \frac{E\,(Ex_j)^2}{nj} - CT$$

$$= \frac{(25000000 + 25000000 + 25000000 + 25000000)}{4} - 25000000$$

$$= 0$$

$$SS.Treatment = \frac{E(Ex_i)^2}{ni} - CT$$

$$= \frac{(25000000 + 25000000 + 25000000 + 25000000)}{4} - 25000000$$

$$= 0$$

Table 105 ANOVA table of antibody titers of mouse anti - tetanus serum during day 90 and 180.

Source	df	SS.	MS	F
Treatment	3	0	0	-
Block	3	0	0	-
Residual	9	0	0	
Total	15	0		

Treatment ; from table, $F_{0.05}$ (3, 9) = 3.86

$0 < F_{0.05}$; Accept the null hypothesis (H_{10}) (P<0.05)

Block ; from table, $F_{0.05}$ (3, 9) = 3.86

$0 < F_{0.05}$; Accept the null hypothesis (H_{20}) (P<0.05)

Hence, there are no significant difference in titer level both among each month period and each time period.

3) Result from table 27 were filled in contingency table 106, 108 and 110

Table 106 Contingency table of antibody titers of mouse anti - tetanus serum during day 0 and 15.

Day\month	0	3	6	9	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
0	10	10	10	10	40	1600	10
3	10	10	10	10	40	1600	10
7	50	50	50	50	200	40000	50
15	250	250	250	250	1000	1000000	250
Ex_i	320	320	320	320	1280		
$(Ex_i)^2$	102400	102400	102400	102400			
$\overline{x_i}$	80	80	80	80			

Calculation data from table 106

$$Ex_{ij}^2 = 260800$$

$$CT = \frac{(E_{ij}\, x_{ij})^2}{N} = \frac{(1280)^2}{(4)(4)} = 102400$$

$$SS.Total = Ex_{ij}^2 - CT$$

$$= 260800 - 102400$$

$$= 158400$$

$$SS.Block = \frac{E\ (Ex_j)^2}{nj} - CT$$

$$= \frac{(1600 + 1600 + 40000 + 1000000)}{4} - 102400$$

$$= 158400$$

$$SS.Treatment = \frac{E(Ex_i)^2}{ni} - CT$$

$$= \frac{(102400 + 102400 + 102400 + 102400)}{4} - 102400$$

$$= 0$$

Table 107 ANOVA table of antibody titers of mouse anti - tetanus serum during day 0 and 15.

Source	df	SS.	MS	F
Treatment	3	0	0	-
Block	3	158400	52800	-
Residual	9	0	0	
Total	15	158400		

Treatment ; from table, $F_{0.05}$ (3, 9) = 3.86

$0 < F_{0.05}$; Accept the null hypothesis (H_{10}) (P<0.05)

Block ; from table, $F_{0.05}$ (3, 6) = 3.86

$0 < F_{0.05}$; Accept the null hypothesis (H_{20}) (P<0.05)

Hence, there are no significant difference in titer level both among each month period and each time period.

Table 108 Contingency table of antibody titers of mouse anti - tetanus serum during day 30 and 75.

Day\month	0	3	6	9	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
30	1250	1250	1250	1250	5000	25000000	1250
45	1250	1250	1250	1250	5000	25000000	1250
60	6250	6250	6250	6250	25000	625000000	6250
75	1250	1250	1250	1250	5000	25000000	1250
Ex_i	10000	10000	10000	10000	40000		
$(Ex_i)^2$	100000000	100000000	100000000	100000000			
$\overline{x_i}$	2500	2500	2500	2500			

Data from table 108

$Ex_{ij}^2 = 175000000$

$$CT = \frac{(E_{ij}\,x_{ij})^2}{N} = \frac{(40000)^2}{(4)(4)} = 100000000$$

$$SS.Total = Ex_{ij}^2 - CT$$

$$= 175000000 - 100000000$$

$$= 75000000$$

$$SS.Block = \frac{E\,(Ex_j)^2}{nj} - CT$$

$$= \frac{(25000000 + 25000000 + 25000000 + 625000000)}{4} - 100000000$$

$$= 75000000$$

$$SS.Treatment = \frac{E(Ex_i)^2}{ni} - CT$$

$$= \frac{(100000000 + 100000000 + 100000000 + 100000000)}{4} - 100000000$$

$$= 0$$

Table 109 ANOVA table of antibody titers of mouse anti - tetanus serum during day 30 and 75.

Source	df	SS.	MS	F
Treatment	3	0	0	-
Block	3	75000000	25000000	-
Residual	9	0	0	
Total	15	0		

Treatment ; from table, $F_{0.05}$ (3, 9) = 3.86

$0 < F_{0.05}$; Accept the null hypothesis (H_{10}) (P<0.05)

Block ; from table, $F_{0.05}$ (3, 9) = 3.86

$0 < F_{0.05}$; Accept the null hypothesis (H_{20}) (P<0.05)

Hence, there are no significant difference in titer level both among each month period and each time period.

Table 110 Contingency table of antibody titers of mouse anti - tetanus serum during day 90 and 180.

Day\month	0	3	6	9	Ex_j	$(Ex_j)^2$	$\overline{x_j}$
30	1250	1250	1250	1250	5000	25000000	1250
45	1250	1250	1250	1250	5000	25000000	1250
60	1250	1250	1250	1250	5000	25000000	1250
75	1250	1250	1250	1250	5000	25000000	1250
Ex_i	5000	5000	5000	50000	20000		
$(Ex_i)^2$	25000000	25000000	25000000	25000000			
$\overline{x_i}$	1250	1250	1250	1250			

Data from table 110

$$Ex_{ij}^2 = 25000000$$

$$CT = \frac{(E_{ij}\,x_{ij})^2}{N} = \frac{(20000)^2}{(4)(4)} = 25000000$$

$$SS.Total = Ex_{ij}^2 - CT$$

$$= 25000000 - 25000000$$

$$= 0$$

$$SS.Block = \frac{E\,(Ex_j)^2}{nj} - CT$$

$$= \frac{(25000000 + 25000000 + 25000000 + 25000000)}{4} - 25000000$$

$$= 0$$

$$SS.Treatment = \frac{E(Ex_i)^2}{ni} - CT$$

$$= \frac{(25000000 + 25000000 + 25000000 + 25000000)}{4} - 25000000$$

$$= 0$$

Table 111 ANOVA table of antibody titers of mouse anti - tetanus serum during day 90 and 180.

Source	df	SS.	MS	F
Treatment	3	0	0	-
Block	3	0	0	-
Residual	9	0	0	
Total	15	0		

Treatment ; from table, $F_{0.05}$ (3, 9) = 3.86

$0 < F_{0.05}$; Accept the null hypothesis (H_{10}) (P<0.05)

Block ; from table, $F_{0.05}$ (3, 9) = 3.86

$0 < F_{0.05}$; Accept the null hypothesis (H_{20}) (P<0.05)

Hence, there are no significant difference in titer level both among each month period and each time period.

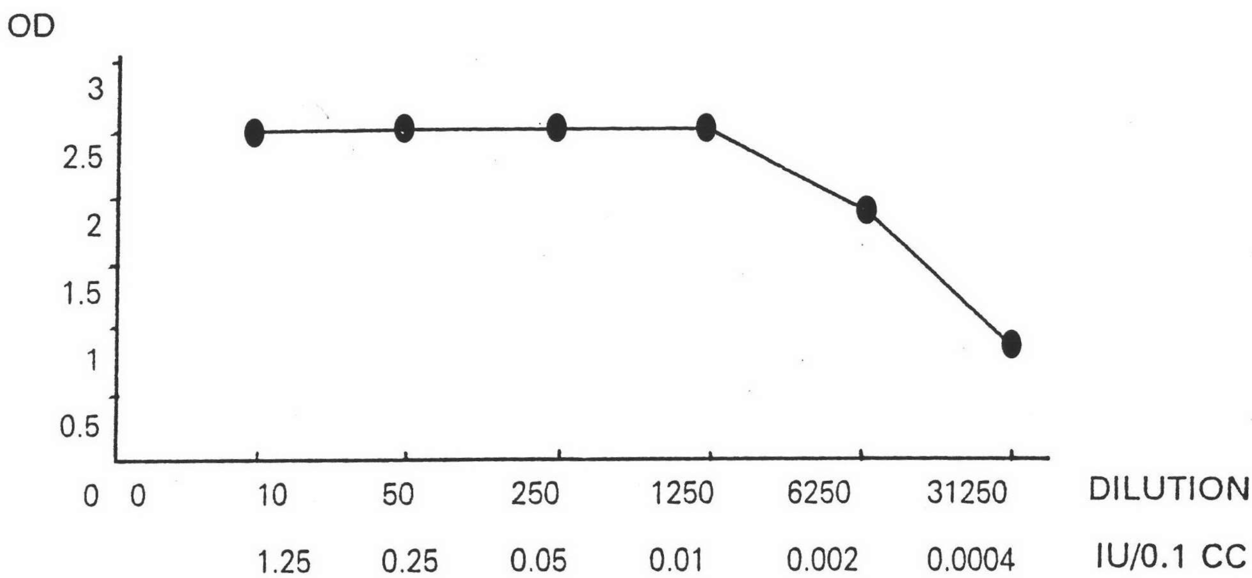

Figure 27 Correlation of OD determined by ELISA and various concentration of human anti-tetanus serum.

* Procedure was similar to ELISA part in method, using goat anti-human IgG as a second antibody.

In this experiment, International Unit (I.U.) was determined as follow : Example ; In TT group, the antibody titers was 1250 and OD was 1.550 (From figure 28 in appendix I)

OD = 1.550

I.U. reading from graph = 0.002

Approximate I.U. of serum was = 1.250 x 0.002 = 2.5 I.U.

In addition for TTM and TT + TTM group the I.U. of serum can be determined in the similar way as above.

In practical, the amount of human anti-tetanus serum used for passive immunization is 250 I.U.

Table 112 F - Ratio for 0.5 (Above) and 0.1 (Below) level of Significance

df2 \ df1	1	2	3	4	5	6	8	12	24	α
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 6234.33	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	4.33 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 8.68	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 3.36	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
α	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 113 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)

VITA

Miss Krisana Sethpiyanon was born on January 31, 1968, in Roi-Et, Thailand. She graduated the Bachelor of Science in Pharmacy in 1991 from the Faculty of Pharmaceutical Science, Khon Kaen University, Khon Kaen, Thailand and the Master of Business Administration major marketing management in 1994 from Dhurakitpundit University, Bangkok, Thailand.