

# Complement Fixation Test with Toxoplasma Antigens Supplied by [REDACTED]

## 1. Preliminary titration of complement in presence of normal and toxoplasma antigens

		<u>Complement 1:30</u>										
		1	2	3	4	5	6	7	8	9	10	
		0.26	0.24	0.22	0.20	0.18	0.16	0.14	0.12	0.1	0.08	0
S	Saline for Saline. 0.74	0.76	0.78	0.80	0.82	0.84	0.86	0.88	0.90	0.92	1.00	
A 2.5cc	Antigen	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	
B	Saline for antig. ser.	0.51	0.53	0.55	0.57	0.59	0.61	0.63	0.65	0.67	0.75	

A = Toxo antigen B = normal

## 2. Sera to be tested

	<u>Dilutions</u>	<u>Ant needed</u>
✓ R. Black (60°C) (13 Dec)	2, 4, 8, 16, 32, 64, 128, 256	1.0 cc of 1:2
✓ Monkey Toxo-lyo (60°) (23 Dec 42)	"	1.0 cc of 1:2
✓ Normal Monkey (progeny) (60°) 11934 Pre-2084/11 47	"	1.0 cc of 1:2
✓ G. pig #1037 - toxo (56°)	16, 32, 64, 128, 256	(0.2cc + 1) 2cc of 1:2

## 3. Antigen needed for test

2.5cc for prelim. complement titration  
 2.5cc " overnight " "  
7.25cc " sera  
 12.25cc

## 4. Complement needed for test

For prelim. test - 5cc of 1:30  
 " overnight test - " "  
 " test - 31cc of 1:?

Complement for preliminary tests on hemolysin etc. - @ 0.5-1cc.

Preliminary Titration of Amboceptor

DEC 15 1947

to be used in Toxoplasma Complement Fixation Test

Amboceptor - old glycerolated stored in refrigerator

Sheep RBC - Bleeding of Dec 10; in Alsever's since Dec. 13.  
Washed 4x Dec. 15

Complement - G. pig frozen in CO<sub>2</sub> box since May 5, 1947

System - 0.25cc ambo. + 0.5cc saline + 0.25cc 3% RBC - 10 min  
then add 0.5cc of complement 1:30

1:1000	44
1:2000	3.5 3.9
1:2500	3 3.5
1:3000	2 3
1:4000	1 2
1:5000	? 1
1:6000	? 1
Saline + Complement	00
Amboc. 1:1000 - no complem.	00

The unit of amboceptor was estimated to be 0.25cc of 1:1500

The sensitized RBC were therefore prepared by mixing equal volumes (63cc) of 3% RBC and 1:500 amboceptor

Titration of Complement - Prelim. Incub. 37°C for 1/2 hr.

	Unit of Complement 1:30									
	0.24	0.22	0.20	0.18	0.16	0.14	0.12	0.10	0.08	0
Saline	2 2	11	1 ±	? 0	0 0	0 0	0 0	0 0	0 0	0 0
Toxo antigen	44	44	3.9 3.9	3.5 3.5	33	22	11	? 1	0 ±	0
Normal "	44	44	3.9 3.9	3.5 3.9	33	22	11	± 1	? ±	0

One exact unit of complement = 0.222cc of 1:30

$$\frac{.44}{30} = \frac{0.5}{x} \quad x = 34 \quad \therefore 0.5cc \text{ of } 1:34 \text{ has 2 units}$$

COMPLEMENT-FIXATION TEST WITH TOXOPLASMA ANTIGEN (WARREN)

DEC 15 1947

<u>TUBE</u>	<u>SERUM</u>	<u>SER. DIL.</u>	<u>ANTIGEN</u>	<u>RESULT</u>
1	[REDACTED]	2	TOXO	3 2-3
2		4		1-2 1-
3		8		0 0
4		16		0 0
5		32		0 0
6		64		0 0
7		128		0 0
8		256		0 0
9	[REDACTED]	2	NCAM	0 0
10		4		0 0
11		8		0 0
12		16		0 0
13		32		0 0
14		64		0 0
15		128		0 0
16		256		0 0
17	[REDACTED]	2	TOXO	4 4-
18		4		4 4-
19		8		4 4-
20		16		4 4-
21		32		3 3
22		64		1 1
23		128		0 0
24		256		0 0
25	[REDACTED]	2	NCAM	2 1-2
26		4		2 2
27		8		1 ±
28		16		0 0
29		32		0 0
30		64		0 0
31		128		0 0
32		256		0 0
33	[REDACTED]	2	TOXO	0 0
34		4		0 0
35		8		0 0
36		16		0 0
37		32		0 0
38		64		0 0
39		128		0 0
40		256		0 0

<u>TUBE</u>	<u>SERUM</u>	<u>SER. Dil.</u>	<u>ANTIGEN</u>	<u>RESULT</u>
41	[REDACTED]	2	NCAM	0 0
42	[REDACTED]	4		0 0
43	[REDACTED]	8		0 0
44	[REDACTED]	16		0 0
45	[REDACTED]	32		0 0
46	[REDACTED]	64		0 0
47	[REDACTED]	128		0 0
48	[REDACTED]	256		0 0
49	[REDACTED]	32	TOXO	4 4-
50	[REDACTED]	64		4 4-
51	[REDACTED]	128		4 4-
52	[REDACTED]	256		0 0
53	[REDACTED]	32	NCAM	0 0
54	[REDACTED]	64		0 0
55	[REDACTED]	128		0 0
56	[REDACTED]	256		0 0
57	[REDACTED]	2	NaCl	0 0
58	[REDACTED]	2	NaCl	0 0
59	[REDACTED]	2	NaCl	0 0
60	[REDACTED]	32	NaCl	0 0

Titration of Complement Simultaneously with Test  
 Refrigerator - - - hours + 37°C for 1/2 hour.

Amount of Complement 1:34

	<u>0.5</u>	<u>0.4</u>	<u>0.3</u>	<u>0.25</u>	<u>0.2</u>	<u>0.15</u>	<u>0.1</u>	<u>0</u>
S Saline	3.9 3.9	3.5 3.9	2 3	1 2	? 1	0 ±	0 ±	0 0
A Toxo Antigen	4 4	4 4	4-? 3.9	3.9 4?	3 3	2 2	1 1	0 0
B Normal "	4 4	4 4	4 4?	3.9 3.9	3 3.5	2 2	1 1	0 0

Sera for C-F tests with Toxoplasma Antigens.

Suspected Cases - Mrs. Florence E. White - Age 32 - Chorioretinitis - H<sub>3</sub>

[Redacted] - 10 months old - Microcephaly  
(Pat. of Dr. Doral) Chorioretinitis (Macula)  
Psychomotor  
retardation

Benger - St  
" - mother } #3  
Van Greveld's patient - Eys - in refrig  
" " mother. Eys - in "

[Redacted] (Czechoslov.) - Cerebral Calcif.  
Neur. dist. #3

Mother  
✓ Norma Scroggins (acute and convul.) - Encephalitis 5/21 - dis  
9/10 - a-4

Other Sera

[Redacted]

[Redacted]

7 days  
26 "  
53 "

Sera Tested

- H<sub>3</sub> 1. [redacted] white - Age 32 yrs. Chorioretinitis - positive skin test
- 2. [redacted] nos. old - Microceph, Chorioret., Psychomot. - pt. of Dr. Doual - California
- 3. [redacted]
- H<sub>3</sub> 4. [redacted] - Neurol. dist.; cerebral calcif. - Czechoslovakia
- H<sub>3</sub> 5. [redacted]
- H<sub>3</sub> 6. [redacted]
- H<sub>3</sub> 7. [redacted]
- 8. [redacted] - pt. of Dr. Van Creveld - Amsterdam, Holland
- { 24 }  
9. [redacted]
- d<sub>5</sub> 10. [redacted] - age 13 - encephalitis unkn. etiology - 8/21/47 ser. - 2 days
- a<sub>4</sub> 11. [redacted] " (cincinnati) - 9/10/47 " - 22 days
- D<sub>1</sub> 12. [redacted] - positive skin test
- ? 13. [redacted] - negative skin test
- 14. [redacted] - pigeon pneumonitis - 7 day serum
- 15. [redacted] " - " - 26 " "
- 16. [redacted] " - " - 53 " "
- 17. Toxo G. pig serum #1037 - positive control

Complement Used - 2 lyophilized STD ampules - 2cc each reconstituted in 3.5 cc distilled H<sub>2</sub>O

Preliminary Titration of 1:30 Complement (0.15cc + 4.35cc saline)  
 1/2 hr. at 37°C - add sensitized RBC → 1/2 hr. at 37°C.

	0.26	0.24	0.22	0.20	0.18	0.16	0.14	0.12	0.10	SALINE
SALINE	44	44	3939	3539	3939	11	01	00	00	00
Toxo Antigen	44	44	44	44	4-42	3939	335	23	22	00

Thus 1 unit of complement in presence of toxo antigen is 0.2cc of 1:30

$$\frac{0.4}{30} = \frac{0.5}{x} \quad \frac{30 \times 0.5}{0.4} = 37.5 \quad \therefore 0.5 \text{ cc of } 1:37.5 = 2 \text{ units}$$

To 3.4cc of the undil. complement was added 123.1cc of saline (this was a mistake - 124.1cc should have been used) to make a 1:37.5 dilution.

### Titration of Complement Simultaneously with Test. Refrigerator

	0.5	0.4	0.3	0.25	0.2	0.15	0.1	0
SALINE	44	44	44	3535	33	11	04	00
Toxo Antigen	44	44	44	44	3535	33	01	00
Normal "	44	44	44	3539	33	22	11	00

TOXOPLASMA COMPLEMENT FIXATION TEST

DEC 30 1947

1

TUBE      SERUM      Ser. dilot.      Antigen

1		2	Toxo	0
✓ 2		4	"	0
✓ 3		8	"	0
✓ 4		16	"	0
✓ 5		32	"	0

6		2	Normal	0
✓ 7		4	"	0
✓ 8		8	"	0

✓ 9		2	Saline	0
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10		2	Toxo	0
11		4	"	0
12		8	"	0
13		16	"	0
14		32	"	0

15		2	Normal	0
16		4	"	0
17		8	"	0

18		2	Saline	0
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19		2	Toxo	0
20		4	"	0
21		8	"	0
22		16	"	0
23		32	"	0

24		2	Normal	1-2
25		4	"	0
26		8	"	0

27		2	Saline	0
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28		2	Toxo	0
29		4	"	0
30		8	"	0
31		16	"	0
32		32	"	0

33		2	Normal	0
34		4	"	0
35		8	"	0

36		2	Saline	0
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TUBE

SERUM

Serum dil.

Antigen

Result

37	2	Toxo	0
38	4	"	0
39	8	"	0
40	16	"	0
41	32	"	0
42	2	Normal	0
43	4	"	0
44	8	"	0
45	2	Saline	0
46	2	Toxo	0
47	4	"	0
48	8	"	0
49	16	"	0
50	32	"	0
51	2	Normal	0
52	4	"	0
53	8	"	0
54	2	Saline	0
55	2	Toxo	0
56	4	"	0
57	8	"	0
58	16	"	0
59	32	"	0
60	2	Normal	0
61	4	"	0
62	8	"	0
63	2	Saline	0
64	2	Toxo	0
65	4	"	0
66	8	"	0
67	16	"	0
68	32	"	0
69	2	Normal	0
70	4	"	0
71	8	"	0
72	2	Saline	0

TOXOPLASMA COMPLEMENT FIXATION TEST

DEC 30 1947 2

TUBE	Serum	Serum dil.	Antigen	Result
73		2	Toxo	0
74		4	"	0
75		8	"	0
76		16	"	0
77		32	"	0
78		2	Normal	0
79		4	"	0
80		8	"	0
81		2	Saline	0
82		2	Toxo	0
83		4	"	0
84		8	"	0
85		16	"	0
86		32	"	0
87		2	Normal	0
88		4	"	0
89		8	"	0
90		2	Saline	0
91		2	Toxo	0
92		4	"	0
93		8	"	0
94		16	"	0
95		32	"	0
96		2	Normal	0
97		4	"	0
98		8	"	0
99		<del>16</del> 2	Saline	0
100		2	Toxo	1+!
101		4	"	0
102		8	"	0
103		16	"	0
104		32	"	0
105		2	Normal	0
106		4	"	0
107		8	"	0
108		2	Saline	0

<u>Tube</u>	<u>SERUM</u>	<u>Serum dil.</u>	<u>Antigen</u>	<u>Result</u>
109		2	Toxo	0
110		4	"	0
111		8	"	0
112		16	"	0
113		32	"	0
<del>114</del>				
114		2	Normal	0
115		4	"	0
116		8	"	0
117		2	Saline	0
118		2	Toxo	0
119		4	"	0
120		8	"	0
121		16	"	0
122		32	"	0
123		2	Normal	0
124		4	"	0
125		8	"	0
126		2	Saline	0
127		2	Toxo	0
128		4	"	0
129		8	"	0
130		16	"	0
131		32	"	0
132		2	Normal	0
133		4	"	0
134		8	"	0
135		2	Saline	0
136		2	Toxo	0
137		4	"	0
138		8	"	0
139		16	"	0
140		32	"	0
141		2	Normal	0
142		4	"	0
143		8	"	0
144		2	Saline	0

DEC 30 1947

<u>Tube</u>	<u>Serum</u>	<u>Dilution</u>	<u>Antigen</u>	
145	G. pig 1037-Toxo	32	Toxo	4
146	"	64	"	4
147	"	128	"	2
148	"	256	"	0
			⋈	
149	"	32	Normal	0
		<del>64</del>		
150	"	32	Saline	0

- Purpose - 1) POTENCY (no. of units) of antigen used for fixation and skin tests  
 2) Preliminary Standardization of Reagents

Toxo Antigen - CAM #3A prepared by Warren on 10/20/47 stored in frozen state (dry ice) as 20% suspension since then.

Thawed - flocculent ppt. centrif. on horizontal at 2,000 rpm for 10 minutes.

Complement - Frozen - Lot of 10-14-48

Preliminary Titration - 37°C for 1/2 hr.; then sensitized RBC and again 37°C - 1/2 hr.

	1	2	3	4	5	6	7	8
	0.3	0.25	0.2	0.18	0.15	0.12	0.10	Saline
<u>Saline</u>	44	44	33.5	12	01	0th	00	00
<u>Toxo Antigen #3A</u>	44	44	44	44	44	3.9	3.9	2-33

0.15cc taken as 1 exact unit for antigen.

$\therefore \frac{0.3}{0.15} = \frac{30}{x}$       0.5cc of complement 1:50 = 2 units

Toxo Guinea Pig Serum 2472 - sent by Warren frozen

Bleeding of 6/21/48 - 34 days after inoculation

C-F titer in Warren's test of 6/23/48 - 1:128 (4+) no aggl.

For present test - serum diluted 1:16 and heated 56°C for 30 min.

$$\begin{array}{r} 0.25 \times \\ \hline 30 \\ \hline 125 \\ 120 \\ \hline 50 \\ \hline \end{array}$$

TUBE				FIXATION
1	Toxo antigen #3A - undiluted (20%)	+ Toxo. g. pig (1:16)		4 4
2	"	1:2	+	4 4
3	"	1:4	+	4 4-
4	"	1:8	+	3 3
5	"	1:16	+	0 0
6	"	1:32	+	0 0
7	Normal CAM. - undil. (20%)	+	"	0
8	Saline	+	"	0
9	Toxo g. pig	1:32	+ Toxo antigen undil.	4 4
10	"	1:64	+	4 4
11	"	1:128	+	4 4
12	"	1:256	+	4 4-
13	"	1:512	+	4 3.5

### Simultaneous Titration of Complement

	Complement 1:50							
	1	2	3	4	5	6	7	8
1.9cc =	0.5	0.4	0.3	0.25	0.20	0.15	0.10	Saline
<u>SALINE</u>	4 4	4 4	3 3	2 2	? 2	0 0	0 0	0 0
<b>A</b> Toxo CAM-20%	4	4	4	4	3.5 3.5	3 2.3	1 1	
<b>B</b> Normal CAM-20%	4	4	4	4	3.5 3.5	2 2	? 1	
<b>C</b> N.G."C" Dengue Antig.	4	4	4	4	3.9 3.9	2 (div)	? 1	0 0
<b>D</b> Yellow Fever Antig.	4	4	4	4	4 <del>4</del>	3 3.5	1 2	0 0

Tubes 1-13 completed at 2 P.M. - left at room temp. until 2:40 PM } In refrigerator  
 Simult. Titration completed at 2:40 PM }

TOXOPLASMA COMPLEMENT FIXATION TEST

OCT 22 1948

SERA - all heated at 60°C for 20 minutes  
TOXO ANTIGEN - # 3A (CAM) - 20% diluted 1:2, i.e. 10%  
Normal " - " " " " " "

TUBE	SERUM	DILUTION	ANTIGEN	FIXATION
1	[REDACTED]	2	Toxo	4 4
2	[REDACTED]	4	"	4 4
3	[REDACTED]	8	"	4 4
4	[REDACTED]	16	"	4 4
5	[REDACTED]	<u>32</u>	"	3.5 3.5
6	[REDACTED]	64	"	tr tr
7	[REDACTED]	128	"	0
8	[REDACTED]	256	"	0
9	[REDACTED]	2	Normal	3 3
10	[REDACTED]	4	"	2 2
10-S	[REDACTED]	2	SALINE	0
11	[REDACTED]	2	Toxo	4 4
12	[REDACTED]	4	"	4 4
13	[REDACTED]	8	"	4 4
14	[REDACTED]	16	"	4 4
15	[REDACTED]	<u>32</u>	"	3.5 3
16	[REDACTED]	64	"	1 1
17	[REDACTED]	128	"	0
18	[REDACTED]	256	"	0
19	[REDACTED]	2	Normal	0
20	[REDACTED]	4	"	0
20-S	[REDACTED]	2	Saline	0
21	[REDACTED]	2	Toxo	4 3.5
22	[REDACTED]	4	"	4 3.5
23	[REDACTED]	8	"	3 3
24	[REDACTED]	16	"	2 2
25	[REDACTED]	32	"	0
26	[REDACTED]	64	"	0
27	[REDACTED]	128	"	0
28	[REDACTED]	256	"	0
29	[REDACTED]	2	Normal	0
30	[REDACTED]	4	"	0
30-S	[REDACTED]	2	Saline	0

32

32

16

OCT 22 1948

TUBE	SERUM	Dilution	ANTIGEN	Fixation	
31		2	Toxo	4	4
32		4	"	4	4
33		8	"	4	4
34		<u>16</u>	"	35	3
35		32	"	0	
36		64	"	0	
37		128	"	0	
38		256	"	0	
39		2	Normal	1	1
40		4	"	0	
40-S		2	Saline	0	
41		2	Toxo	4	4
42		4	"	4	4
43		8	"	4	4
44		<u>16</u>	"	4	4
45		<u>32</u>	"	1	1
46		64	"	0	
47		128	"	0	
48		256	"	0	
49		2	Normal	0	
50		4	"	0	
50-S		2	SALINE	0	
51		2	Toxo	4	4
52		4	"	4	3.5
53		<u>8</u>	"	4	3
54		16	"	1	1
55		32	"	0	
56		64	"	0	
57		128	"	0	
58		256	"	0	
59		2	Normal	1	1
60		4	"	0	
60-S		2	Saline	0	
61		2	Toxo	4	4
62		4	"	4	4
63		<u>8</u>	"	4	3.5
64		16	"	1	1
65		32	"	0	
66		64	"	0	
67		128	"	0	
68		256	"	0	

16

16

8

8

OCT 22 1948

TUBE	SERUM	DILUTION	ANTIGEN	FIXATION
69	[REDACTED]	2	Normal	0
70	[REDACTED]	4	"	0
70-S		2	Saline	0
71	[REDACTED]	2	Toxo	0
72	[REDACTED]	4	"	0
73	[REDACTED]	8	"	0
74	[REDACTED]	16	"	0
75	[REDACTED]	32	"	0
76	[REDACTED]	64	"	0
77	[REDACTED]	128	"	0
78	[REDACTED]	256	"	0
79		2	Normal	0
80		4	"	0
80-S		2	SALINE	0
81	[REDACTED]	2	Toxo	4
82	[REDACTED]	4	"	4
83	[REDACTED]	8	"	4
84	[REDACTED]	16	"	4
85	[REDACTED]	32	"	4
86	[REDACTED]	64	"	1
87	[REDACTED]	128	"	0
88	[REDACTED]	256	"	0
89		2	Normal	0
90		4	"	0
90-S		2	SALINE	0
91	[REDACTED]	2	Toxo	4
92	[REDACTED]	4	"	4
93	[REDACTED]	8	"	4
94	[REDACTED]	16	"	4
95	[REDACTED]	32	"	1
96	[REDACTED]	64	"	0
97	[REDACTED]	128	"	0
98	[REDACTED]	256	"	0
99		2	Normal	0
100		4	"	0
100-S		2	SALINE	0

0

32

16

OCT 22 1948

TUBE	SERUM	DILUTION	ANTIGEN	FIXATION	
101		2	Toxo	4	4
102		<u>4</u>	"	3.5	3
103		8	"	0	
104		16	"	0	
105		32	"	0	
106		64	"	0	
107		128	"	0	
108		256	"	0	
109		2	Normal	0	
110		4	"	0	
110-S		2	SALINE	0	
111		4	Toxo	4	4
112		8	"	4	4
113		16	"	4	4
114		<u>32</u>	"	4	4-
115		64	"	1	1
116		128	"	0	
117		256	"	0	
118		4	Normal	0	
119		4	Saline	0	
120		4	Toxo	4	4
121		8	"	4	4-
122		<u>16</u>	"	3.5	3
123		32	"	1	1
124		64	"	0	
125		128	"	0	
126		256	"	0	
127		4	Normal	3	2
128		4	Saline	0	
129		<u>16</u>	Toxo	3.5	3
130		32	"	0	
131		64	"	0	
132		128	"	0	
133		256	"	0	
134		16	Normal	0	
135		16	Saline	0	

4

32

16

16

	0.5	0.4	COMPLEMENT 1:50					no Comp.	
Toxo ANTIGEN	4	4	0.3	0.25	0.20	0.15	0.10	Saline	
	4	4	4	44	3.5	3.9	23	? 2	00

Toxo Complement Fixation Test

1. To determine whether centrifugation at 13,000 rpm for 1 hour in cold will remove component in Normal Chorioallant. Membrane which reacts with some human sera
2. Does Centrifugation at 13,000 rpm affect the number of antigenic units in the toxo antigen?
3. Potency of other toxo antigens

<u>Tube</u>	<u>Serum</u>		<u>ANTIGEN</u>	<u>FIXATION</u>	
1	[REDACTED]	1:2	Normal ORIGINAL	0 +tv	
2		1:4	" " "	0 0	
3		1:2	Normal - 13,000	0 0	
4		1:4	" "	0 0	
5		1:2	Saline	0 0	
6		1:2	Normal - ORIGINAL	0 0	
7		1:2	" - 13,000	0 0	
8		1:2	SALINE	0 0	
9		" "	1:4	Normal - original	1 1
10		" "	1:4	Normal - 13,000	0 0
11	Gpis 2472 (heated 60°C 20min.)	1:16	Toxo 3A - Original - undil (10%)	4 4	
12	"	"	" " 1:2	4 4	
13	"	"	" " 1:4	3 3-	
14	"	"	" " 1:8	0 0	
15	"	"	Toxo 3A - 13,000 - undil (10%)	4 4	
16	"	"	" " 1:2	4 4	
17	"	"	" " 1:4	2 2	
18	"	"	" " 1:8	0 0	
19	"	"	Toxo 3A - Lyophiliz. - undil (20%)	4 4	
20	"	"	" " 1:2	4 4	
21	"	"	" " 1:4	4 4	
22	"	"	" " 1:8	4 4	
23	"	"	" " 1:16	1-2 2-	
24	"	"	Toxo 1C - frozen - undil (20%)	4 4	
25	"	"	" " 1:2 10%	4 4	
26	"	"	" " 1:4 5%	4 4	
27	"	"	" " 1:8 2.5%	4 4	
28	"	"	" " 1:16 1.25%	4 4	
29	"	"	SALINE	0 0	
30	Saline		Toxo 3A - Lyoph. - undil	0 0	
31	"		" 1C - frozen - undil.	0 0	

# SIMULTANEOUS TITRATION of COMPLEMENT

Complement 1:50

	0.5	0.4	0.3	0.25	0.2	0.15	0.10	0
SALINE	44	44	44	33.5	22	? <sup>tr</sup>	00	00
A Normal CAM-13,000	44	44	44	<del>44</del>	3.539	2.53	21	
B Rift Valley Fever Brain	4	4	4	44	44	3.535	22	

COMPLEMENT USED: Tube 4 of Lot (10-14-48); 1:50 dilution - 0.5cc 2 units?

RBC - Lot of Oct. 25 (in Atsever's); sensitized Nov. 1 - 1:500

## TOXO ANTIGENS:

1C - frozen since Oct. 6, 1947; thawed to-day and centrifuged on horizontal.

3A - lyophilized - since Oct. 20, 1947; same lot as 3A frozen kept in ordinary refrigerator until to-day. Reconstituted with 20cc of distilled water - went into solution well except for slight amt which was less than in frozen. Same centrifugation however did not yield as clear a supernatant liquid.

3A - 13000 - Antigen 3A - previously thawed from frozen state <sup>and used in tests</sup> was centrifuged on Sorvall for 1 hour at 13,000 rpm. Su-used

Normal - 13,000 - Normal CAM antigen used in previous tests centrif. at 13,000 - 1 hr. on Sorvall angle in cold room.

TOXOPLASMA COMPLEMENT FIXATION TEST

NOV 2 1948

Sera of Various Patients Used.

Toxo antigen - Pool of frozen & thawed #3A and Lyophilized #3A reconstituted. 10% - @ 4 units

Complement - Tubes 5 to 6 (Lot 10-14-48)

TUBE	SERUM	Dilution	ANTIGEN	FIXATION
1	[REDACTED]	2	Toxo	4 4
2	[REDACTED]	4	"	4 4
3	[REDACTED]	8	"	4 4
4	[REDACTED]	16	"	4 4
5	[REDACTED]	32	"	2 2
6	[REDACTED]	64	"	0
7		2	NCAM	0-fts.
8		2	Saline	0
9	Rainey, <sup>cord</sup> at birth	2	Toxo	4 4
10	7-18-48	4	"	4 4
11		8	"	4 4
12		16	"	4-3
13		32	"	to to
14		64	"	0
15		2	NCAM	0
16		2	Saline	0
17	[REDACTED]	2	Toxo	4 4
18	[REDACTED]	4	"	4 4-
19	[REDACTED]	8	"	2-3 2
20	[REDACTED]	16	"	0
21	[REDACTED]	32	"	0
22		2	NCAM	0
23		2	Saline	0
24	[REDACTED]	2	Toxo (0.27cc antiq.)	4 4
25	[REDACTED]	4	"	2 2
26	[REDACTED]	8	"	0
27	"	16	"	0
28	"	2	NCAM	0
29	"	2	Saline	0

<u>TUBE</u>	<u>SERUM</u>	<u>DILUTION</u>	<u>ANTIGEN</u>	<u>FIXATION</u>
30		2	Toxo	4 4
31		4	"	4 4
32		8	"	4 4
33		16	"	4 4
34		32	"	4 4
35		64	"	4 4
36		128	"	4 4
37		2	NCAM	0
38		2	Saline	0
39		2	Toxo	4 4
40		4	"	4 4
41		8	"	4 4
42		16	"	4 4
43		32	"	2-3 2
44		64	"	0
45		128	"	0
46		2	NCAM	0
47		2	Saline	0
48		2	Toxo	4
49		4	"	3
50		8	"	1
51		2	NCAM	4
52		2	Saline	AC
53		2	Toxo	4 4
54		4	"	4 4
55		8	"	4 4
56		16	"	4 4
57		32	"	4 4
58		2	NCAM	2-3 2.5
59		2	Saline	0
60		2	Toxo	4 4
61		4	"	4 4
62		8	"	4 4
63		16	"	4 4
64		2	NCAM	1 1.2
65		2	Saline	1 1

Toxo Complement Fixation Test

NOV 2 1948

TUBE	SERUM	DILUTION	ANTIGEN	FIXATION
66	[REDACTED]	4	Toxo	4
66-A	[REDACTED]	8	"	4
66-B	[REDACTED]	16	"	4
10 67	[REDACTED]	32	"	4
68	[REDACTED]	64	"	3
69	[REDACTED]	128	"	0
70	[REDACTED]	4	NCAM	4
71	[REDACTED]	4	SALINE	AC
72	[REDACTED]	2	Toxo	4 4
11 73	[REDACTED]	4	"	4 4
74	[REDACTED]	8	"	4-4
75	[REDACTED]	16	"	1 1
76	[REDACTED]	32	"	0
77	[REDACTED]	64	"	0
78	[REDACTED]	2	NCAM	0
79	[REDACTED]	2	SALINE	0
80	[REDACTED]	2	Toxo	4 4
12 81	[REDACTED]	4	"	3 3
82	[REDACTED]	8	"	1 1
83	[REDACTED]	16	"	0
84	[REDACTED]	2	NCAM	0
85	[REDACTED]	2	Saline	0
13 86	[REDACTED]	2	Toxo	4-4
87	[REDACTED]	4	"	1 1
88	[REDACTED]	8	"	0
89	[REDACTED]	16	"	0
90	[REDACTED]	2	NCAM	0
91	[REDACTED]	2	Saline	0
14 92	[REDACTED]	2	Toxo	0
93	[REDACTED]	4	"	0
94	[REDACTED]	8	"	0
95	"	2	NCAM	0
96	"	2	Saline	0

<u>TUBE</u>	<u>SERUM</u>	<u>DILUTION</u>	<u>ANTIGEN</u>	<u>FIXATION</u>
97		2	Toxo	4 4
98		4	"	4 4
99	"	8	"	4 4
100	"	16	"	4-4
101	"	32	"	2-3 2
102	"	64	"	tr tr
103	"	2	NCAM	0
104	"	2	Saline	0
105		2	Toxo	4 4
106		4	"	4 4
107	"	8	"	4 4
108	"	16	"	4 4
109	"	32	"	4 4-
110	"	64	"	1 1
111	"	2	NCAM	2?2
112	"	2	Saline	0?tr?
113		UNDIL.	Toxo	0
114		2	"	0
115	"	4	"	0
116	"	8	"	0
117		UNDIL.	NCAM	0
118		UNDIL.	SALINE	0
119		UNDIL.	Toxo	0
120		2	"	0
121	"	4	"	0
122	"	8	"	0
123		UNDIL.	NCAM	0
124		UNDIL.	Saline	0
125		2	Toxo	0
126		4	"	0
127	"	8	"	0
128	"	16	"	0
129	"	32	"	0
130		2	NCAM	0
131		2	Saline	0

# Toxo Complement Fixation Test

NOV 2 1948

<u>TUBES</u>	<u>SERUM</u>	<u>Dilution</u>	<u>Antigen</u>	<u>Fixation</u>
132	[REDACTED]	2	Toxo	4 4
133	[REDACTED]	4	"	3 3.5
134	"	8	"	0
20 135	"	16	"	0
136	"	32	"	0
137	"	2	NCAM	0
138	"	2	Saline	0
139	[REDACTED]	2	Toxo	0
21 140	[REDACTED]	4	"	0
141	"	8	"	0
142	"	16	"	0
143	"	2	NCAM	0
144	"	2	Saline	0
145	[REDACTED]	2	Toxo	4 4
146	[REDACTED]	4	"	3? 4-
22 147	"	8	"	1-2 2
148	"	16	"	tr
<del>148</del>	"			
149	"	2	NCAM	3 4
150	"	2	Saline	0

	<u>Complement 1:50 (Hemolysis)</u>						<u>Saline</u>
<u>Toxo Antigen Pooled 3A</u>	<u>0.4</u>	<u>0.3</u>	<u>0.25</u>	<u>0.2</u>	<u>0.15</u>	<u>0.1</u>	
	4	4	4	3.5 3.5	1-2 2	0 1	0 0

All sera heated at 60°C for 20 minutes  
 Mixtures in refrigerator at 4:45 P.M.  
 Out of fridge " " 8:45 A.M.

TOXOPLASMA COMPLEMENT FIXATION TEST

NOV 3 1948

<u>TUBE</u>	<u>SERUM</u>	<u>Dilution</u>	<u>Antigen</u>	<u>FIXATION</u>
1		2	Toxo	0
2		4	"	0
3		8	"	0
4		2	NCAM	0
5		2	Saline	0
6		2	Toxo	4 4
7		4	"	4-3,5
8		8	"	3 3
9		2	NCAM	4 4
10		2	Saline	0
11		2	Toxo	4 4
12		4	"	4 4
13		8	"	3 3
14		2	NCAM	4 4
15		2	Saline	4 4
16		2	Toxo	0
17		4	"	0
18		8	"	0
19		2	NCAM	0
20		2	Saline	0
21		2	Toxo	0
22		4	"	0
23		8	"	0
24		2	NCAM	0
25		2	SALINE	0

<u>Tube</u>	<u>SERUM</u>	<u>Dilution</u>	<u>Antigen</u>	<u>Fixation</u>
26		2	Toxo	0
27		4	"	0
28		8	"	0
29		2	NCAM	0
30		2	Saline	0
31		2	Toxo	0
32		4	"	0
33		8	"	0
34		2	NCAM	0
35		2	Saline	0
36		2	Toxo	0
37		4	"	0
38		8	"	0
39		2	NCAM	0
40		2	Saline	0
41		2	Toxo	3 3
42		4	"	3 3
43		8	"	1-2 2
44		2	NCAM	0
45		2	Saline	0
46		2	Toxo	0
47		4	"	0
48		8	"	0
49		2	NCAM	0
50		2	Saline	0
51		2	Toxo	0
52		4	"	0
53		8	"	0
54		2	NCAM	0
55		2	Saline	0

See repeat  
tel on 1/8

Toxo COMPLEMENT FIXATION TEST (contin.)

NOV 3 1948

<u>TUBE</u>	<u>SERUM</u>	<u>Dilution</u>	<u>Antigen</u>	<u>Fixation</u>
56		2	Toxo	4 <del>4</del>
57		4	"	2 <del>2</del>
58		8	"	0
59		2	NCAM	0
60		2	Saline	0
61		2	Toxo	3 4
62		4	"	2 2
63		8	"	0
64		2	NCAM	3 3
65		2	Saline	3 2
66		2	Toxo	0
67		4	"	0
68		8	"	0
69		2	NCAM	0
70		2	Saline	0
71		2	Toxo	0
72		4	"	0
73		8	"	0
74		2	NCAM	0
75		2	Saline	0
76		2	Toxo	4 4
77		4	"	3 4
78		8	"	3 4-
79		2	NCAM	0
80		2	Saline	0
81		2	Toxo	0
82		4	"	0
83		8	"	0
84		2	NCAM	0
85		2	Saline	0

<u>TUBE</u>	<u>SERUM</u>	<u>Dilution</u>	<u>Antigen</u>	<u>Fixation</u>
86		2	Toxo	0
87		4	"	0
88		8	"	0
89		2	NCAM	0
90		2	Saline	0
91		2	Toxo	0
92		4	"	0
93		8	"	0
94		2	NCAM	0
95		2	Saline	0
96		2	Toxo	0
97		4	"	0
98		8	"	0
99		2	NCAM	0
100		2	Saline	0
101		2	Toxo	4 <sup>3.5</sup>
102		4	"	0
103		8	"	0
104		16	"	0
105		32	"	0
106		2	NCAM	0
107		2	Saline	0
108		2	Toxo	1-2?
109		4	"	1
110		8	"	0
111		2	NCAM	0
112		2	SALINE	0
113		2	Toxo	4 <sup>4</sup>
114	"	4	"	4 <sup>4</sup>
115	"	8	"	4 <sup>4</sup>
116	"	16	"	4 <sup>4</sup>
117	"	32	"	1
118	"	2	NCAM	0
119	"	2	Saline	0

Toxo Comp. Fixation (continued)

NOV 3 1948

<u>TUBE</u>	<u>SERUM</u>	<u>Dilution</u>	<u>Antigen</u>	<u>Fixation</u>
120		2	Toxo	0
121		4	"	0
122		8	"	0
123		16	"	0
124		2	NCAM	0
125		2	Saline	0
126		2	Toxo	0 to 2+
127		4	"	0
128		8	"	0
129		2	NCAM	0
130		2	SALINE	0
131		2	Toxo	4 4
132		4	"	4 4
133		8	"	4 4
134		16	"	4 4
135		32	"	4 4-
136		2	NCAM	0
137		2	SALINE	0
138		2	Toxo	4 4
139		4	"	4 4
140		8	"	4 4
141		16	"	4-3.5
142		32	"	0 0
143		2	NCAM	0
144		2	Saline	0
145		Indic. 2	Toxo	4
146		<del>2</del> 4	"	4
147		<del>4</del> 8	"	4
148		<del>8</del> 16	"	4
149		<del>16</del> 32	"	4
<del>150</del>		<del>PR</del>		
150		2	NCAM	4
151		<del>2</del> 4	"	4
153		2	Saline	4
154		4	"	4

1.25 needed

# Simultaneous Titration of Complement 1:50

Toxo Antigen  
Pooled #3 A

<del>0.3</del>	<u>0.3</u>	<u>0.25</u>	<u>0.2</u>	<u>0.15</u>	<u>Saline</u>
	4	4	3.539	2.25	00

# TOXOPLASMA COMPLEMENT FIXATION TEST

NOV 8 1948

- Purpose - 1) To determine whether centrif. at 13,000 rpm. removes component reacting with some normal.  
 2) Does centrif. at 13,000 rpm of toxo antigen affect the titer of positive sera.

An aliquot of each "original" antigen used here was centrifuged on 11/6/48 at 13,000 rpm in the cold room on the Sorvall machine. Tiny pellets formed.

<u>Tube</u>	<u>SERUM</u>	<u>Dilution</u>	<u>Antigen</u>	<u>Fixation</u>
1	[REDACTED]	16 + 32	Toxo # 3A - orig.	4 3.5
2		32 8 64	"	tr 0
3		64 1 128	"	"
4		128 2 256	"	"
5	[REDACTED]	8 <del>128</del> 16	" - 13,000	4 3.5
6		16 32	"	tr 0
7		32 64	"	0
8		64 128	"	0
9		128 256	"	0
10	[REDACTED]	2	NCAM - orig.	2-3 2
11		2	" - 13,000	0 0
12		2	Saline	0 0
13	[REDACTED]	8	Toxo # 3A - orig.	4 4
14		16	" "	4 4-
15		32	" "	3 3-
16		64	" "	0
17	[REDACTED]	8	" - <del>13,000</del>	4 4
18		16	" "	4 4
19		32	" "	3 2.5
20		64	" "	0
21		4	NCAM - orig.	0
22	4	SALINE	0	
23	[REDACTED]	2	Toxo # 3A - orig.	4 4
24		4	" "	3 3
25		8	" "	1-2 1.5
26		16	" "	0
27	[REDACTED]	2	" - 13,000	tr tr
28		4	" - "	0 0
29		8	" - "	0 0

TUBE	SERUM	DILUTION	ANTIGEN	FIXATION
30		2	NCAM-orig	4 4
31		4	"	2 2
32	"	2	NCAM-13,000	0 0
33	"	4	"	0 0
34	"	2	Saline	0 0
<del>35</del>		<del>2</del>	Toxo 3A-orig.	
36		4	"	4 4
37	"	8	"	2 2
38	"	16	"	0
<del>39</del>	"	<del>2</del>	" - 13,000	
40	"	4	"	0 0
41	"	8	"	0 0
42	"	2	NCAM-orig	4 4
43	"	4	" "	3 2
44	"	8	" "	1 1
45	"	2	" - 13,000	0 0
46	"	4	" "	0 0
47	"	8	" "	0 0
48	"	2	Saline	0
49		2	Toxo #3A-orig.	0
50		4	"	0
51		8	"	0
52		2	" - 13,000	0
53		4	" "	0
54		8	" "	0
55		2	NCAM-orig	0
56		2	" - 13,000	0
57		2	Saline	0
58	Gpig 2472	1:16	Toxo-1C-orig. - undil. (10%)	4 4
59	"	"	2	4 4
60	"	"	4	4 4
61	"	"	8	4 4-
62	"	"	16	0
62A	"	"	32	0

Toxo Complement Fixation Test. (contin)

NOV 8 1948

<u>TUBE</u>	<u>SERUM</u>	<u>Dilution</u>	<u>Antigen</u>	<u>Fixation</u>
63	Gpig 2472	1:16	Toxo IC-13,000-Und (10%)	4 ✓
64		"	2	4 ✓
65		"	4	4 ✓
66		"	8	3 3-
67		"	16	0
68		"	32	0
69		"	Saline	0
70		2	Toxo IC-13,000-5%	0
71		4	"	0
72		8	"	0
73		16	"	0
74		2	NCAM - 13,000	0
75		2	Saline	0
76		2	Toxo IC-13,000-5%	0
77		4	"	0
78	Dr. Sunderman	8	"	0
79		16	"	0
80		32	"	0
81		64	"	0
82		2	NCAM - 13,000	0
83		2	Saline	0
84		2	Toxo IC-13,000-5%	0
85		4	"	0
86		8	"	0
87		16	"	0
88		32	"	0
89		64	"	0
90		2	NCAM - 13000	0
891		2	Saline	0
92		2	Toxo IC-13,000-5%	0
93		4	"	0
94		8	"	0
95		2	NCAM - 13,000	0
96		2	Saline	0

NOV 8 1948

<u>TUBE</u>	<u>SERUM</u>	<u>Dilution</u>	<u>ANTIGEN</u>	
97		1:2	Toxo /c - 13,000-5%	4 4-
98		4		3 3
99		8		0
100		16		0
101		2	<sup>CAM</sup> <del>NCAE</del> - 13000	0
102		2	Saline	0
<del>103</del>		<del>2</del>	Toxo /c - 13000-5%	
104		4	"	4 4
105		8	"	4 3.5
106		16	"	0
107		4	NCA M - 130	0
		2		
108		4	Saline	0

Simultaneous Titration of Complement 1:50

Toxo Antigen # / C	<u>0.3</u>	<u>0.25</u>	<u>0.2</u>	<u>0.15</u>	<u>Saline</u>
Centrif. - 13,000 - 1 hr. 5%	4	4	4 3.9	3 3.5	0 0

Mixtures completed at 4 P.M. + in refrigerator @ 4:30 P.M.  
 " removed from refrigerator at 8:30 AM 11/9/48  
 RBC added and in water bath (37°C) by 9 AM

NOV 8 1948 - 4:30 PM

Check on Activity of Complement 1:50

<u>Saline</u>	<u>0.5</u>	<u>0.4</u>	<u>0.3</u>	<u>Saline</u>
	4	4	3.5	0

# Patients with Congenital Toxoplasmosis AND/OR THEIR MOTHERS Diagnosis by Dye Test

No.	NAME	Age	Dye test titer	(-F)	
1			B-8-3 1:4,096	0!!	✓
1-M			" 1:4,096	1:32	
2		s.	" 1:1,024	1:32	(Normal 1:4)
3		s. also CSF	" 1:4,096	1:32	
3-M			" 1:1,024	1:16	(Normal 1:4)
3-F			" 1:1,024	1:16	
4		2.	B-9-9 1:4,096	1:32	
4-			" 1:1,024	1:16	
5		yrs.	(B-8-5) 64-1:128	1:8	
5-			" 64-1:256	1:8	
6		os. also CSF	1:1,024	1:16	
6-			(B-8-4) "	1:4	
7		s. Zuelzer case	B-9-3 1:1,024	1:16	
7-		after being born	" 1:1,024	1:16	
8		ears after 2 1/2 days of red care	3/1/1948 (B-8-5) 1:256	1:8	
9		4/6/1944	" 1:1,024	1:64	
9-		yrs. after being born	1:256	1:4	
10		15 mos	B-9-6 1:16,384	1:128 +?	
10		"	" 1:1,024	1:32	
10		7 mos.	1:2 ?; neg 1:4	Ac	
11		6 1/2 yrs.	B-9-9 1:64	1:16	
11			" 1:64	1:32	
12		10 days	1:256	1:32	
12-M			1:1,024	1:32	

C-F

32

32

0

32

64

1:8

1:8

1:2

M

# Toxoplasma C-F Antigen from Mice

Mouse Brain -

20% suspension in saline - freeze + thaw 3x and

allow to stand in refrigerator overnight

Centrifuge 13,000 for 1/2 hr.

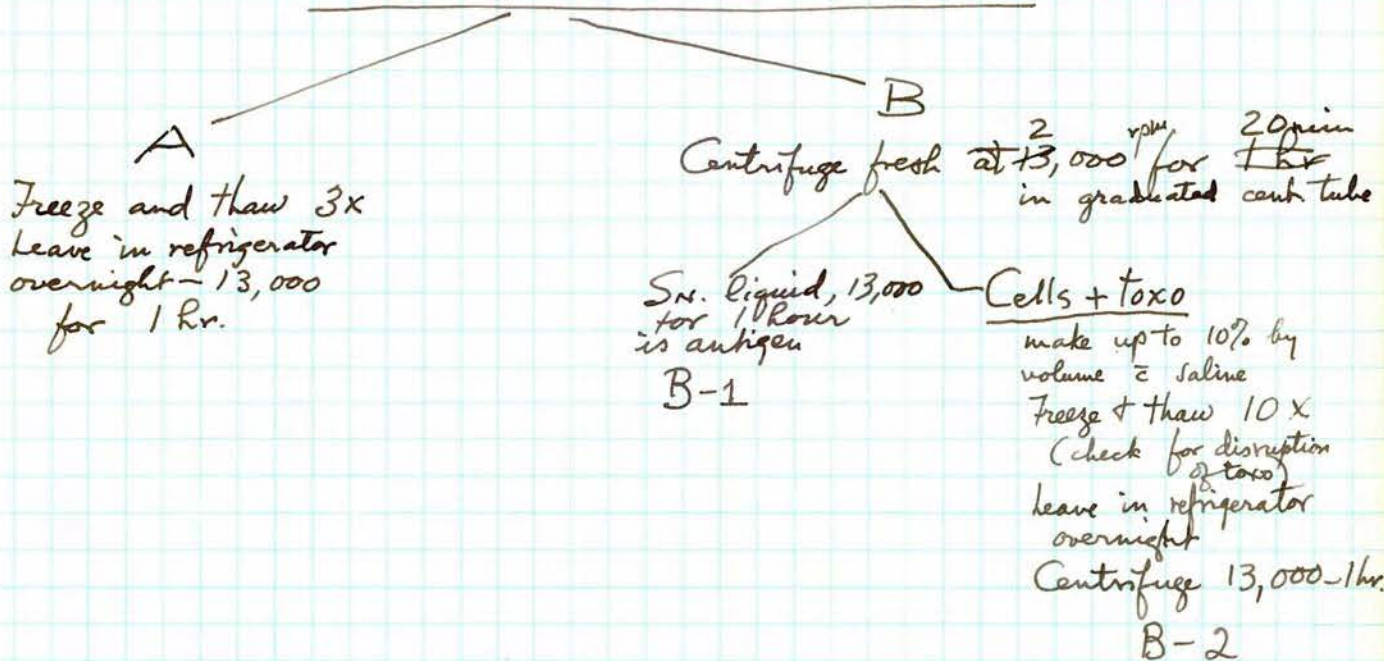
Sn. liquid - 13,000 for 1 hour.

Test sn. liquid for a) anticomplementary properties

b) No. of antigenic units with  
toxog. guinea pig sera

## Mouse PERITONEAL EXUDATE

### FRESH PERITONEAL EXUDATE



Toxoplasma C-F Antigen in Infected Mouse Brain

NOV 9 1948

5 P.M. - 18 mice - about 2-3 weeks old - each received 1 <sup>cc</sup> cor.  
0.03 cc of 3-day peritoneal exudate from mice,  
diluted 1:2 in saline. Toxoplasma numerous but  
not overabundant.

NOV 10 1948 - all mice appear well

NOV 11 1948 - 9 A.M. - 3 dead, 1 having convulsions died,  
others showing varying degrees of hyperexcit. to  
convulsions. This is the earliest I have ever  
seen CNS signs due to Toxoplasma.

Film of brain of 1 dead mouse - loaded with  
large Toxoplasma - no bacteria seen.

2 dead mice put in refrigerator - others watched.

3 P.M. - 3 more died and others definite  
CNS signs.

4 P.M. - remaining mice killed & exsanguinated.

17 mouse brains weighed 6.45 Gm. ground with  
saline without sand and made up to 32.2 cc  
to make a 20% suspension. Put into a  
250 cc Pyrex reagent bottle - quickly frozen  
on sides of bottle by rolling in dry ice - alcohol  
mixture. Quickly thawed at 37°C. Process  
repeated 3 times. Culture on BAP - 00

Tissue suspension put in refrigerator at 5:15 P.M.

NOV 12 1948

9 A.M. - 0.5 cc removed - inoculated in 2-3 wk old  
mice i.c.v. to check on death of Toxoplasma and  
absence of other infectious agent

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
5 D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	

Discarded  
at 6 weeks  
all were

Centrifugation at 13,000 rpm. (small angle) - in cold room

1<sup>st</sup> spin - 30 min. - 2.2 cc of clear su. liquid obtained  
2<sup>nd</sup> spin - 1 hour - small translucent pellet in each tube.

21.7 cc of very clear <sup>th.</sup> hemoglobin-stained fluid  
obtained - added 0.22 cc of mercurate 1:100  
to yield fin. conc. of 1:10,000

4 Dec from 20 miles =  $\frac{16 \times 4}{20}$

3 P.M. - 0.64cc of exudate from 5-day mouse - fair number of toxo - not abundant - diluted 1:10 in saline and immediately inoculated into 30 mice, each weighing @ 20 Gm. Dose - 0.2cc

NOV 12 1948 - all mice O.K.

NOV 13 1948 - " " O.K.

NOV 14 1948 - " " O.K.

NOV 15 1948 - all mice O.K. - many, but not all, exhibit distended bellies.

All mice killed with chloroform - abdominal skin resected and peritoneal exudate removed with capillary pipette. Material from 2 mice discarded because blood got into it from ruptured vessel. Viscous, cloudy + fibrin strands.

Total exudate collected from 28 mice - 33cc

Count of 1:100 dil. = 19 million toxo/cc undil.

Approximately 1cc of saline added to abdom. of each mouse and additional 28cc collected -

Count of 1:100 dil. = 10 million toxo/cc undil.

The original exudate and the washings were pooled.

Fraction A - 21cc (in 2 Lueroid tubes) frozen and thawed in (dry-ice alcohol) and 37°C water bath - 3 times  
After second freezing - fibrin coagulum appeared in each tube. Left in refrigerator overnight.

Fraction B - 40cc of pooled exudate (10cc in each graduated centrif. tube) centrifuged on horizontal at @ 2,000 rpm for 20 min.  
Sediment - 0.1cc by volume in each tube - total 0.4cc.

" - Supernatant liquid - centrifuged on angle at 13,000 rpm for 1 hour in cold. No definite pellet but sides probably sl. coated. Sn. liquid very clear removed - 0.4cc merthiolate 1:1000

added to yield final conc. of 1:10,000.  
Stored in refrigerator.

Fraction B - Cells + toxoplasma - taken up in 8cc saline transferred to Ten Broeck grinder and ground to smooth suspension - quite cloudy. Tubes washed out with 8cc of saline and added to original - to yield a final 2.5% suspension (1:40) by volume.

Examination of 1 drop  $\bar{c}$  methylene blue showed masses of toxoplasma and almost equal "mass" by volume of leukocytes. Distributed in 2 leucocoids and frozen and thawed 10X. Considerable flocculation appeared. At end of process examination with methylene blue still revealed some intact toxoplasmas (with cytoplasm unstained) but it was evident that most of toxoplasma had disintegrated. Stored in refrigerator overnight.

1:40 - final dilution 1:320  
1:64

# Test for Anticomplementary and Complement-Enhancing Activity of Various Toxoplasma Antigens of Mouse Origin

11/12/48

		Complement 1:50						
		0.5	0.3	0.25	0.20	0.15	0.10	Saline
S	Saline		4+	4+	4+	3.5	2-3	0
U	20% Mouse Brain		4+	4+	3.9	2-3	1-2	0
A	Perit. Exud.-A - undil.		0	0	0	0	0	0
B	" " - 1:2		0	0	0	0	0	0
C	" " - 1:4		0	0	0	0	0	0
D	Perit. Exud.-B <sub>1</sub> - undil.		0	0	0	0	0	0
E	" " " - 1:2		0	0	0	0	0	0
F	" " " - 1:4		0	0	0	0	0	0
G	Perit. Exud.-B <sub>2</sub> - undil. 1:40		4-3	3.5	12	0	0	0
H	" " - 1:2 1:80		4+	3.5	2-3	1-2	0	0
I	" " - 1:4 1:160		4+	4+	3.5	2-2	1-1	0

## Brief description of antigens

- 20% mouse brain - 20% susp. in saline, frozen and thawed 3x, overnight in refrig., 13,000 rpm for 30 min + then for 1 hour.
- Perit. Exud. "A" - Whole exudate (+ washings), frozen + thawed 3x, overnight in refrig., 2000 rpm. for 10 min., sn. 13,000 rpm for 1 hour
- Perit. Exud. "B<sub>1</sub>" - Exud. centrif. 2000 rpm. for 20 min. Sn liquid used 13,000 rpm. for 1 hour
- Perit. Exud. "B<sub>2</sub>" - Toxoplasma + cells leukocytes (from B<sub>1</sub>) - 2.5% suspension. Frozen + thawed 10x; overnight in refrig., 2000 rpm for 10 min; sn. 13,000 rpm for 1 hour.

Test 1 - Complement (Lusteroid previously used & refrozen - Lot of 10/14/48)  
1:50 in saline - prev. titer 0.4 of 1:50 for saline

RBC - Lot of 10/25/48 in Alsever's - good condition  
same as used in other tests

Comp 1:50					<u>Saline</u>
<u>0.5</u>	<u>0.4</u>	<u>0.3</u>	<u>0.25</u>		
4	4	4	4		0

Since no endpoint was obtained (0.4nc expected) it was thought that the cells might have become more susceptible to hemolysis. Fresh cells obtained from General (bled 11/15/48)

Test 2

Complement 1:50				<u>Saline</u>
<u>0.4</u>	<u>0.3</u>	<u>0.25</u>	<u>0.20</u>	
4	4	4	4-?	0

Since ~~again~~ the same result was obtained, the RBC of 10/25/48 were apparently not to blame, and it was thought desirable to check on whether too much amboceptor might have been used by mistake.

Test 3

- RBC of 11/16 again washed and sensitized with ~~RBC~~ 1:500 amboceptor as usual.

New lot of complement (of 11/13/48) also tested for comparison - designated as N; other lot as O

	Complement 1:50					<u>Saline</u>
Old lot	<u>0.4</u>	<u>0.3</u>	<u>0.25</u>	<u>0.2</u>	<u>0.15</u>	
	4 <sup>+</sup>	4 <sup>+</sup>	4 <sup>+</sup>	3.93.9	3.3	0
New lot	4 <sup>+</sup>	4 <sup>+</sup>	4 <sup>+</sup>	4.14-	3.3	

All the sensitized RBC appeared densely agglutinated - not as usual.

# Toxo Complement Fixation Test

NOV 17 1948

Complement - 1 tubercoid of 10/14/48 - part removed for preliminary test rest refrozen & used later

RBC - 11/15/48 lot in Alsever's

Preliminary Test - mixtures prepared and sensitized RBC added immediately and incubated at 37°C for 30 min

COMMERCIAL  
SALINE USED

## Complement 1:50

	1	2	3	4	5	6	0
	0.4	0.3	0.25	0.20	0.15	0.10	
S SALINE	44	44	3.5.9	223	1?1	0tr	00
A Haw. Dengue #10	44	44	44	44	23	01	
B Toxo Mouse Brain (20%)	44	44	44	3.9.9	23	1?1	
C Toxo Per. Exd. - B <sub>2</sub> - 1:4	44	44	44	33	2.2	01	
D " " " - B <sub>1</sub> - 1:16	44	44	44	33	2-32	0tr	

## Potency of Various Toxo Antigens

Behavior of Serum <sup>(Protein reaching)</sup> NCAM to Toxo Mouse Brain (13,000)

De Felice Sera

- All sera heated at 60°C for 20 min

TUBE	Serum	Antigen	Fixation
1	Toxo G. pig #2472 - 1:64	Toxo CAM-1C-13,000-10% und.	4 4
2	"	5 1:2	4 4
3	"	2.5 1:4	4 3
4	"	1:8	tr
5	"	1:16	0
6	"	1:32	0
7A	- "	Saline	0
7B	- "	Normal Mouse Brain 20%	0
8	" 1:64	Toxo Mouse Brain - 20% und.	4 4
9	"	10% 1:2	4 4
10	"	5% 1:4	4 4
11	"	2.5 1:8	3 3
12	"	1:16	0
13	"	1:32	0
14	"	1:64	0
15	" 1:64	Toxo Per Exd. - B <sub>1</sub> - 1:16	4 4
16	"	1:32	4 4
17	"	1:64	4 4
18	"	1:128	tr tr

TUBE	SERUM	Antigen	Fixation
19	Toxo g. pig 2472 - 1:64	Toxo Per Saut. - B <sub>2</sub> - 1:4 0.65	4 4
20	"	1:8 0.3%	4 4
21	"	1:16	2? 2?
22	"	1:32	0
23	"	1:64	0
24	[REDACTED]	Toxo KC-13000-5%	0
25	[REDACTED]	"	0
26	[REDACTED]	"	0
27	[REDACTED]	"	0
28	[REDACTED]	NCAM-13,000-5%	0
29	[REDACTED]	Saline	0
30	[REDACTED]	Toxo I-C-13,000-5%	0
31	[REDACTED]	"	0
32	[REDACTED]	"	0
33	[REDACTED]	"	0
34	[REDACTED]	NCAM-13,000-5%	0
35	[REDACTED]	Saline	0
36	[REDACTED]	Toxo Mouse Brain-20%	4 3
37	[REDACTED]	"	1 1
38	[REDACTED]	"	0
39	[REDACTED]	"	0
40	[REDACTED]	Normal Mouse Br.-20%	0
41	[REDACTED]	Saline	0

Titration of Complement 1:50 - simultaneously  $\bar{c}$  Test

	0.4	0.3	0.25	0.2	0.15	0.10	0
S Saline	44	44	3.535	12	01	00	00
B Toxo antig. - P.E.-B <sub>2</sub> (1:4)	44	44	3.939	33	12	0tw	
C " " - " - B <sub>1</sub> (1:16)	44	439	325	22	?1	00	
A! " " - 20% Mouse Br.	44	44	44-	3.939	223	0?2	
D Gpig serum 2472 (1:64)	44	44-	3.939	3.53	22	6tw	

Mixtures in refrig. at 4:00 P.M.  
auto " at 8:30 AM

Toxo C-F Test

NOV 19 1948

On Nov. 18

The 20% Toxo Mouse Brain antigen was again centrifuged for 1 hour at 13,000 rpm to see whether it would remove reactivity with Mrs. Prattini's serum

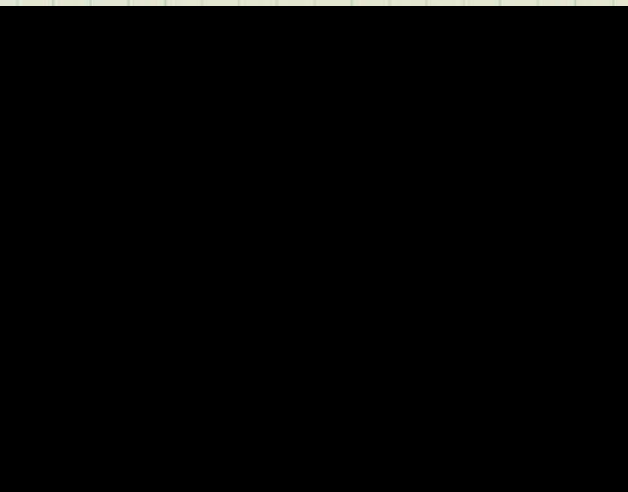
PE-B-1 antigen - centrifuged for 1 hr. at 13,000 again to see if it would ~~improve~~ remove ~~its capacity to~~ the last bit of anticomplementary property  
 PE-A antigen - same

<u>Tube</u>	<u>Serum</u>	<u>Dilution</u>	<u>Antigen</u>	<u>Fixation</u>
149	Prattini, Mrs.	2	Recentrif. Toxo M.Br.	4 4
150	" "	2	20% Normal M.Br. (new - 2 cycles, 13,000)	4 4
151	" "	2	PE-B1 (Recentrif.) (1:16)	2 3
152	" "	2	PE-B2 (orig.) 1:4	1-2 2-3

Complement 1: <sup>75</sup>/<sub>0</sub>

	<u>0.5</u>	<u>0.4</u>	<u>0.3</u>	<u>0.25</u>	<u>0.2</u>	<u>0.15</u>	<u>Saline</u>
S Saline	4	4	3.5 3.5	2-3 2	? tw	0 tw	0 0
A Recentrif. Toxo Mouse Br.		4	4	4 4	3.5 3.5	2 2	
B Recentrif. PE-B1 1:8		1 1	0 tw	0 0	0 0	0 0	
C " " " 1:16		4 4	2-3 2.5	1-2 1-2	0 tw	0 0	
D Recentrif. A 1:16		4 4	3.3	2 1-2	0 1	0 tw	

To



at

- Family

test potency of Allantoinic kind  
Repeat potency on Mouse Br.  
+ other extracts

50%  
Selenium - Used  
by mistake

DEC 16 1948

# Antigenicity Patency of Several Toxoplasma Preparations - New and Repeat.

New - Allantoic Fluid from Toxo Infected Eggs - 17 day Harvest

Repeat - Using 1:16 S. pig. Toxo Serum repeat tests  
 Toxo CAM #1C (centrif. at 13,000 rpm to-day)  
 Toxo Mouse Brain  
 Toxo PE - B<sub>1</sub>  
 Toxo PE - B<sub>2</sub>

0.5 cc of Complement (lot #13/48) 1:33.3 used = 2 3 units on basis of 2.16  
 2 1.9 units on basis of selenium

Tube	SERUM	Dil.	ANTIGEN	DIL.	FIXATION
1	Toxo. g. pig #2463	1:16	Toxo CAM #1C	UND. (10%)	4 4
2	"	"	"	2	2 2
3	"	"	"	4	0 0
4	"	"	"	8	0 0
5	"	"	"	16	0 0
6	"	1:16	Toxo Allant. fl.	UNDIL.	0
7	"	"	"	2	0
8	"	"	"	4	0
9	"	"	"	8	0
10	"	"	"	16	0
11	"	"	"	32	0
12	"	"	Normal Allant. fl.	UNDIL.	0
13	" 0.27 cc	1:16	Toxo Mouse Brain (20%)	2	2 2-?
14	"	"	"	4	0
15	"	"	"	8	0
16	"	"	"	16	0
17	"	"	"	32	0
18	"	1:16	Toxo PE - B <sub>1</sub>	16	4 4-
19	"	"	"	32	0 2
20	"	"	"	64	0
21	"	"	"	128	0
22	"	"	"	256	0
23	"	1:16	Toxo PE - B <sub>2</sub>	4	4 4
24	"	"	"	8	2-3 2.5
25	"	"	"	16	0 0
26	"	"	"	32	0 0
27	"	32	Toxo CAM #1C - 10%	4	4 4
28	"	64	"	4	3 3.5
29	"	128	"	1	0 0
30	"	256	"	0	0 0
31	"	512	"	0	0 0
32	"	1024	"	0	0 0

STANDARD B&W "NEE" 317

Complement 1:33.3

Compl. 1:50

G.p. serum 1:16

0.3      0.25      0.20  
 44      44      44

Saline - <sup>0.5 0.4 0.3 0.2 0.2 0.15</sup> 4425100  
 Normal Allant. Fluid - 444-3.5

DEC 17 1948

Repeat Test Using Complement 1:50

Tube	Serum	Dilut.	Antigen - Dilut.	Fixation
1	Toxo gpig 2463	1:16	Toxo CAM#1 (10%) 2	4 4
2	"	"	" 4	4 4
3	"	"	" 8	0 0
4	"	"	Toxo Allant. Und	0 0
5	"	"	" 2	0 0
6	"	"	Toxo Mouse Br. 2	4 4
7	"	"	" 4	3.5 3.5
8	"	"	" 8	0 0
9	"	1:32	Toxo CAM#1-Und.	4 4
10	"	1:64	"	4 4
11	"	1:128	"	4-4
12	"	1:256	"	2 2
13	"	1:512	"	0 0
14	"	1:1024	"	0 0

Comp 1:50 (overnight)

5% Dextrose-saline (by mistake)

0.5   0.4   0.3   0.25   0.20   0.15   0  
 44   3.94-   2.535   22   00   00   0  
 44   4-4   3.535   23

Sap B #2 (used in another test)

2467  
 2468

# Toxoplasma C-F Test

DEC 20 1948

<u>Tube</u>	<u>Serum</u>	<u>Dil.</u>	<u>Antigen</u>	<u>Fixation</u>
✓ 1.	[REDACTED]	4	Toxo CAM #K	4 4
2	[REDACTED]	8	" (10%)	4 4
3	[REDACTED]	16	"	2-3 2
4	[REDACTED]	32	"	0
5	[REDACTED]	64	"	0
6	[REDACTED]	4	NCAM (10%)	0
7	[REDACTED]	4	Saline	0
✓ 8	[REDACTED]	4	Toxo CAM #C	4 4
9	[REDACTED]	8	"	4 4
10	[REDACTED]	16	"	2 2
11	[REDACTED]	32	"	0
12	[REDACTED]	64	"	0
13	[REDACTED]	4	NCAM	0
14	[REDACTED]	4	Saline	0
✓ 15	[REDACTED]	2	Toxo CAM	tr tr
16	[REDACTED]	4	"	0
17	[REDACTED]	8	"	0
18	[REDACTED]	2	NCAM	0
19	[REDACTED]	2	Saline	0
✓ 20	[REDACTED]	4	Toxo CAM	4 4
21	[REDACTED]	8		4 4
22	[REDACTED]	16		4 3.5
23	[REDACTED]	32		2 2
24	[REDACTED]	64		0
25	[REDACTED]	128		0
26	[REDACTED]	4	NCAM	0
27	[REDACTED]	4	Saline	0
✓ 28	[REDACTED]	8	Toxo	4 4
29	[REDACTED]	16		4 3.5
30	[REDACTED]	32		0
31	[REDACTED]	64		0
32	[REDACTED]	8	NCAM	0
33	[REDACTED]	8	Saline	0

12618  
2657  
58

STANDARD B & P "No. 100"

<u>Tube</u>	<u>Serum</u>	<u>Dilut.</u>	<u>Antigen</u>	<u>Fixation</u>
✓ 34		2	Toxo	4 4
35		4	"	4 3.5
36		8	"	0 0
<del>37</del>				
37		2	NCAM	0
38		2	Saline	0
✓ 39		2	Toxo	0
40		4	"	0
41		8	"	0
42		2	NCAM	0
43		2	Saline	0
✓ 44		2	Toxo	
<del>45</del>		4	"	4 4
46		8	"	4 4
47		16	"	4 3
48		32	"	0 $\frac{3}{2}$
49		64	"	0
50		4	NCAM	0
51		4	Saline	0
✓ 52		2	<del>Toxo</del>	
53		4	"	4 3.5
54		8	"	0 0
55		16	"	0
56		32	"	0
57		64	"	0
58		4	NCAM	0
59		4	Saline	0
✓ 60		2	Toxo	0
61		4	"	0
62		8	"	0
63		2	NCAM	0
64		2	Saline	0

Toxo C-F Test (continued)

DEC 20 1948

Tube	Serum	Dilution	Antigen	Fixation
✓ 65	[redacted]	2	Toxo	3 2.5
66	[redacted]	4	"	0 0
67	[redacted]	8	"	0
68	[redacted]	16	"	0
69		2	NCAM	0
70		2	Saline	0
✓ 71	[redacted]	2	Toxo	4 4
72	[redacted]	4	"	4 4-
73	[redacted]	8	"	0 0
74	[redacted]	16	"	0
75		2	NCAM	0
76		2	Saline	0
✓ 77	[redacted]	2	Toxo	0
78	[redacted]	4	"	0
79	[redacted]	8	"	0
80	[redacted]	16	"	0
81		2	NCAM	0
82		2	Saline	0
✓ 83	[redacted]	2	Toxo	4-4-
84	[redacted]	4	"	2 2
85	[redacted]	8	"	0
86	[redacted]	16	"	0
87		2	NCAM	4 4-
88		2	Saline	4 4-
89	[redacted]	2	Toxo	0
90	[redacted]	4	"	0
91	[redacted]	8	"	0
92	[redacted]	16	"	0
93		2	NCAM	0
94		2	Saline	0

Dye test neg.

STANDARD B&P "NOTEPAPER"

Tube	Serum	Dil.	Antigen	Dil.			
95	Toxo G. pig # 2467	4	Toxo #1C (10%)	2	4	4	
96		"		4	3	2.5	
97		"		8	tr	tr	
98		"		16	0		
99		"	"	Toxo Alkaut. Fluid	UND	2	2.5
100		"	"	"	2	0	
101		"	"	Norm. All. Fl.	UND.	0	
102		"	"	Toxo M. Br. (20%)	4	4	4
103		"	"	"	8	2	2
104		"	"	"	16	tr	0
105	"	"	"	32	0		
106	4.25	"	PE-B <sub>1</sub>	32	4	4	
107		"		64	2.3	2.5	
108		"		128	tr	tr	
108A		"		256	0		
109	4.5 needed	"	PE-B <sub>2</sub>	8	4	4	
110	"	16		4	4		
111	"	32		tr	tr		
112		8	Toxo #1C 10%	undil.	4	4	
113		16		"	4	4	
114		32		"	4	4	
115		64		"	4	4-	
116		128		"	3	3	
117		256		"	0		
117A		4		Saline	0		
118	[Redacted] (Repeat)	4	Toxo #1C		4	4	
119		8		3.5	3		
120		16		0	0		
121		32		0			
122		4	NCA-M		0		
123		4	Saline		0		
124A	[Redacted]	2	Toxo #1C		4	4	
124		4		tr	tr		
125		8		0	0		
126		16		0			
127		32		0			
128		42	NCA-M		0	0	
129		42	Saline		0	0	

STANDARD B & P "NORWAY"

Tube	Serum	Dil.	Antigen	Fixation
130	[REDACTED]	2	Toxo #/C <del>NCAM</del>	0
131	[REDACTED]	4		0
132	[REDACTED]	8		0
133	[REDACTED]	16		0
134	[REDACTED]	2	NCAM	0
135	[REDACTED]	2	Saline	0
136	[REDACTED]	2	Toxo #/C	0
138	[REDACTED]	4	"	0
138	[REDACTED]	8	"	0
139	[REDACTED]	2	NCAM	0
140	[REDACTED]	2	Saline	0
141	[REDACTED]	2	Toxo #/C	0
142	[REDACTED]	4	"	0
143	[REDACTED]	8	"	0
144	[REDACTED]	2	NCAM	0
145	[REDACTED]	2	Saline	0
146	[REDACTED]	2	Toxo #/C	4 4
147	[REDACTED]	4	"	1.5 1.5
148	[REDACTED]	8	"	0
149	[REDACTED]	2	NCAM	0
150	[REDACTED]	2	Saline	0

SIMULT. Titration of Complement 1:50

	0.5	0.4	0.35	0.3	0.25	0.2	0.15
SALINE	4	4	4	4	3.93.9	2.25	0.5
Toxo #/C				4	4	4	2.2

<u>Tube</u>	<u>Serum</u>	<u>Dilution</u>	<u>Antigen</u>	<u>Fixation</u>
154		2	Toxo #1C	0
155		4		0
156		8		0
157		16		0
158		32		0
159		2	Normal CRM	0
160		2	Saline	0
161		2	Toxo #1C	0
162		4		0
163		8		0
164		16		0
165		32		0
166		2	Normal	0
167		2	Saline	0
168		2	Toxo #1C	0
169		4		0
170		8		0
171		16		0
<del>172</del>	no complement	<del>32</del>		
173		2	Normal CAT1	0
174		2	Saline	0

TABLE V

Persistence of Complement-Fixing Antibody in Children with Clinically Recognized Congenital Toxoplasmosis and in Mothers without Recognized Signs of Infection except for Having Given Birth to Infected Children.

Children with Congenital Toxoplasmosis				Mothers of Children with Proved Infection			
No.	NAME	AGE	C-F Titer	No.	NAME	TIME SINCE DELIVERY	C-F Titer
1		5 weeks	0	1		5 weeks	1:32
2		10 "	1:32	2		10 "	1:32
		18 "	1:32			18 "	1:16
3		6 months	1:32	3		6 months	1:16
4		9 "	1:16	4		9 "	1:4
5		14 "	1:32				
6		15 "	1:32	5		15 "	1:16
7		15 "	1:128+?	6		15 "	1:32
8		15 "	1:2	7		24 "	1:4
		30 "	1:2			30 "	1:4
9		3 1/2 years	1:8	8		3 1/2 years	1:8
10		3 2/3 "	1:16	9		3 2/3 "	1:4
11		5 "	1:16	10		5 "	1:16
12		6 "	1:8	11		6 "	1:8
13		6 1/2 "	1:16	12		6 1/2 "	1:32
14		7 "	0				
15		7 1/2 "	1:2				
16		11 "	1:4	13		11 "	1:8
				14		6 weeks	1:64
						4 years	1:8
				15		9 8 months	1:32
						<del>30</del> "	1:16
						18 "	
				16		8 "	1:32
				17	F-45	3 years	1:64



Time after delivery

	0	1:2	1:4	1:8	1:16	1:32	1:64	1:128
0 - 11 months	1				1	3		
12 - 23 "		1				2	1	
24 - 35 "		1						
36 - 47 "				1	1			
48 - 59 "								
60 - 71 "					1			