CHAPTER V

METHOD USED IN THE TEST

From the studies on the proposed method, the developed method are as follows.

1. Add 0.2 ml of DNA working solution (4 mg/100 ml) to each well of polystyrene plate. Incubate for 1 hour at 37 C.

2. Wash 3 times with 0.1 M potassium phosphate buffer pH 7.0 containing 1% BSA or 1% gelatin.

3. Add 0.2 ml of 1:200 diluted normal rabbit serum in phosphate albumin buffer. Incubate 1 hour 1 at 37 C. Wash as before.

4. Add 0.2 ml of two fold serial dilution of test serum to each well starting at 1:8. Incubate for 1 hour at 37 C. Wash as described before.

5. Add 0.2 ml of 1:100 conjugate to each well. Incubate for 1 hour at 37 C. Wash the plate as previously described.

6. Add 0.2 ml of substrate to each well, observe the reaction or stop reaction with 1% sodium azide at 10 minutes.

The criteria for differentiate results:

positive = the well that develops brown color

negative = the well that does not develop brown color

control = the well that is not exposed to test sera, this well should be negative

the end point titer = the highest dilution of serum that develops color.

TABLE 1 TEST FOR OPTIMAL CONDITION FOR INCUBATING DILUTED CONJUGATE

Temp.	Time (hrs.)	Titers	
		Positive serum	Negative serum
4 C	18	256	16 *
RT.	3	256	16 *
RT.	4	256	32
RT.	18	512	64
37 C	½	256	16 *
37 C	1	256	16 *
37 C	2	512	32
37 C	3	512	64

* optimal condition for incubating diluted conjugate

TABLE 2. TEST FOR OPTIMAL TIME FOR INCUBATING SUBSTRATE

Time (Minutes)	Positive					Negative					Background
	32	64	128	256	512	8	16	32	64	128	
1	W	-	-	-	-	-	-	-	-	-	-
5	2+	1+	W	-	-	W	1+	-	-	-	-
10	4+	3+	1+	-	-	2+	1+	-	-	-	- *
15	4+	4+	2+	1+	-	2+	2+	1+	-	-	-
20	4+	4+	3+	2+	1+	3+	3+	2+	1+	-	1+
30	4+	4+	3+	3+	2+	4+	3+	2+	2+	1+	2+

* optimal time for incubating substrate

TABLE 3. OPTIMAL CONDITION FOR INCUBATING DILUTED UNKNOWN SERA

Temp.	Time (hrs.)	Titers	
		Positive serum	Negative serum
4 C	18	256	64
RT.	3	128	16
RT.	4	dark background	dark background
RT.	18	dark background	dark background
37 C	½	128	8
37 C	1	256	16 *
37 C	2	256	32
37 C	3	512	64

* optimal condition for incubating diluted unknown sera

TABLE 4 pH OF BUFFER USED FOR WASHING PLATE

pH	Titres		Background
	Positive serum	Negative serum	
6.0	256	64	1+
6.5	256	32	-
7.0	256	16	- *
7.5	256	16	- *
8.0	128	8	-

* optimal pH of buffer used for washing plate

TABLE 5 EFFECT OF PROTEIN USED IN THE WASHING BUFFER

PROTEIN IN BUFFER	Titers		Background
	Positive serum	Negative serum	
0.5% BSA	256	32	-
1% BSA	256	16	- *
2% BSA	256	32	1+
3% BSA	256	32	1+
0.5% gelatin	256	64	-
1% gelatin	256	16	- *
2% gelatin	64	32	-
3% gelatin	128	32	-

* optimal concentration of protein used in the washing buffer

TABLE 6 EFFECT OF WASHING PROCESS WITH BUFFER, DISTILLED WATER, TAP WATER.

WASHING TECHNIC	Titers		Background
	Positive	Negative	
Buffer 3 times	256	16	- *
Buffer 2 time, and Dist. water 1 time	256	16	- *
Buffer 1 time, and Dist. water 2 times	128	32	-
Dist. water 3 times	64	16	-
Tap water 3 times	The color is too dark to read.		

* suitable condition for washing process.

TABLE 7 THE STABILITY OF DNA COATED PLATE

No.	Titers					
	1 month	2 month	3 month	4 month	5 month	6 month
1	512	512	512	512	256	256
2	1024	1024	512	256	256	256
3	1024	1024	1024	512	256	128
4	1024	512	1024	512	256	256
5	256	256	256	128	128	64
6	16	16	16	16	8	8
7	16	8	8	neg	neg	neg
8	neg	neg	neg	neg	neg	neg
9	8	8	neg	neg	neg	neg
10	neg	neg	neg	neg	neg	neg