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Evaluation of Wassermann Reaction (W.R.), Kahn and Cardiolipin tests on sera inactivated at 56°C. for 30 minutes and 60°C. for 3 minutes

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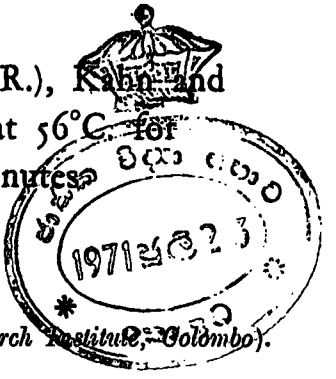

About 175 to 200 samples of bloods are examined daily for evidence of syphilis at the Medical Research Institute, Colombo. The sera are inactivated at 56°C. for 30 minutes. As the samples tested are fairly large, heating of sera for 30 minutes consumes much time.

Rytz (1935) inactivated sera at 60°C. for 3 minutes. Rein et al (1940) found that sera heated at 63°C. for 3 minutes and at 69·5°C. for one minute gave the same results as sera heated at 56°C. for 30 minutes. Neurath et al (1947) inactivated at 63°C. for three minutes and Kline (1948) heated sera between 60 to 63°C. for three to five minutes. With a view of shortening the time of inactivation it was proposed to test the efficacy of the results of W.R., Kahn and Cardiolipin tests on sera inactivated at 60°C. for three minutes and at 56°C. for 30 minutes.

The blood samples from the Male Venereal Diseases Clinic, General Hospital, Colombo were used for checking the Wassermann Reaction, Kahn and Cardiolipin results. The Clinical diagnosis was supplied by the Venereal Diseases Clinic authorities.

The technique used for performing the Cardiolipin test is described here as it is a comparatively new test. Kline's (1946) technique with slight modification was adopted for doing the flocculation test. The method briefly is as follows: Wax rings of 14 m.m. diameter were made on glass slides 2 × 3". Eight rings were made on each slide. Antigen is made by mixing (1) distilled water 0·85 ml. (2) 1% alcoholic solution of cholestrol 1 ml. (3) antigen 0·1 ml. and (4) 0·85% sodium chloride 2·45 ml. After each addition the mixture is well shaken. It is used ten minutes after preparation and discarded after one hour. Cardiolipin antigen was obtained from Messrs. La Motte Chemical Products Co., Baltimore, U.S.A. It was stored below 12°C.

The sera are added with a capillary pipette standardised to the 55th hole of Starret's Gauge and antigen pipette is standardised to the 70th hole. The volumes delivered by these pipettes are 0·025 and 0·008 ml. respectively. However before use they are tested for accuracy by measuring the volume of 300-500 drops. Two drops of serum (0·05 ml.) are added and then one drop of antigen (0·008 ml.) is added. Known positive and negative sera are added as controls. The slides are placed in a wooden tray and rotated at a speed of 180 r.p.m. in a circle of 2" diameter for four

minutes or until the controls give the desired results. The results are read with the naked eye with the usual notations :— + + + +, + + +, + +, + and 0. The test is repeated in cases where the sera are spilt out of the rings.

The comparative results of the various tests are shown in Tables 1 to 6 inclusive.

TABLE 1

W.R.—Results of Sera inactivated at 56°C. per half hour and 60°C. per 3 minutes.

Inactivation						
Temperature	*Time*	*Total*	0	±	+	+ +
56°C.	30 min.	1067	633 59·2%	55 5·2%	87 8·2%	292 27·4%
60°C.	3 min.	1067	643 60·2%	47 4·4%	86 8·1%	291 27·3%

At 56°C. there is a slight increase in W.R. ± results than at 60°C. but W.R. + and + + results are the same for both temperatures.

TABLE 2

Kahn—Results of Sera inactivated at 56°C. per half hour and 60°C. per 3 minutes.

Inactivation						
Temperature	*Time*	*Total*	0	+	+ +	+ + + *and over*
56°C.	30 min.	1067	800 68·7%	66 6·2%	184 17·3%	83 7·8%
60°C.	3 min.	1067	794 67·6%	73 6·8%	190 17·8%	83 7·8%

There is hardly any difference between the Kahn results of sera inactivated at 56°C. for 30 minutes and at 60°C. for 3 minutes.

TABLE 3

Cardiolipin—Results of Sera inactivated at 56°C. per half hour and 60°C. per 3 minutes.

Inactivation						
Temperature	*Time*	*Total*	0	+	+ +	+ + + *and over*
56°C.	30 min.	1067	619 58·1%	91 8·5%	216 20·2%	141 13·2%
60°C.	3 min.	1067	616 57·8%	89 8·3%	219 20·3%	145 13·6%

Here again there is no difference between the results of the sera inactivated at 56°C. for 30 minutes and at 60°C. for 3 minutes.

It could be concluded from the serological point of view without considering the clinical diagnosis that there is hardly any difference between W.R., Kahn and Cardiolipin results of sera inactivated at 56°C. for 30 minutes and at 60°C. for 3 minutes.

Tables 4 and 5 show the serological and clinical results.

TABLE 4

W.R.—Results compared with the Clinical State.

Inactivation		Syphilitic					Non-Syphilitic				
Temp.	Time	Total	0	±	+	+ +	Total	0	±	+	+ +
56°C.	30 min.	360	140 38·9%	26 7·2%	40 11·1%	154 42·8%	231	174 75·3%	10 4·3%	18 7·8%	29 12·6%
60°C.	3 min.	360	146 40·5%	15 4·2%	42 11·7%	157 43·6%	231	174 75·3%	16 6·9%	16 6·9%	25 10·9%

Syphilitic sera give more W.R. ± results at 56°C. than at 60°C., but W.R. + and + + results are the same for both temperatures. At 60°C. there are less W.R. + and + + results in non-syphilitic cases than at 56°C.

TABLE 5

Kahn—Results compared with the Clinical State.

Inactivation		Syphilitic					Non-Syphilitic				
Temp.	Time	Total	0	+	+ +	+ + + and over	Total	0	+	+ +	+ + + and over
56°C.	30 min.	360	179 49·6%	19 5·3%	105 29·2%	57 15·9%	231	208 90%	3 1·3%	18 7·8%	2 0·9%
60°C.	3 min.	360	179 49·6%	18 5·0%	106 29·5%	57 15·9%	231	205 88·7%	5 2·2%	19 8·2%	2 0·9%

There is very little difference between the syphilitic and non-syphilitic sera inactivated at 56°C. and at 60°C.

TABLE 6

Cardiolipin—Results compared with the Clinical State.

Inactivation		Syphilitic					Non-Syphilitic				
Temp.	Time	Total	0	+	+ +	+ + + and over	Total	0	+	+ +	+ + + and over
56°C.	30 min.	360	123 34·1%	33 9·2%	110 30·6%	94 26·1%	231	205 88·8%	7 3·0%	10 4·3%	9 3·9%
60°C.	3 min.	360	123 34·1%	33 9·2%	110 30·6%	94 26·1%	231	205 88·8%	7 3·0%	10 4·3%	9 3·9%

Cardiolipin results are the same for syphilitic and non-syphilitic cases for both temperatures.

Conclusion

The specificity and sensitivity are the same for Kahn and Cardiolipin tests for sera inactivated at 56°C. for 30 minutes and 60°C. for 3 minutes. W. R. gives slightly less false positive results at 60°C. that at 56°C.

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