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*Meinicke (Kvittingen) and Mueller Ballung (Clotting) tests in comparison with Standard Kahn and Cardiolipin (Kline) tests

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Introduction

Since the introduction of seroreactions in the diagnosis of syphilis by Wassermann et al. (1906) numerous authors have contributed their research to improve sensitivity and specificity of these reactions.

In this connections Mueller (1926) described a special antigen to give specific clottings with syphilitic sera.

To shorten the time interval of seroreactions several new reactions and modifications were reported. Kvittingen (1948) reported a modification of the techniques using Meinicke antigen (Chediak, Meinicke, Meinicke—Fischer, Izikowitsch) as being a rapid and reliable slide test.

The trends of the serologists are to adopt simpler methods which yield reliable results in the shortest possible time. Meinicke (Kvittingen), Mueller Ballung (Clotting), Kahn (1928) and Cardiolipin (Kline, 1947) tests were done on 15,057 sera to ascertain which of these tests satisfied the above conditions.

The latter two tests are performed as routine tests at the Medical Research Institute, Colombo. The antigens were supplied by Messrs. Astra Soedertalje, Sweden, (Meinicke Kvittingen test), the Federal Serum Institute, Vienna, Austria, (Mueller Ballung test), Messrs. La Motte Co., Baltimore (Kline test). The Kahn antigen was prepared locally accordingly to Kahn's prescriptions from dried beef heart (Difco).

Methods

Meinicke test : 10 ml. of 3·5 per cent. saline are placed in a test tube in a water bath of 56°C for 15 minutes. At the end of the first 10 minutes 1 ml. of Meinicke antigen is warmed up similarly for the last 5 minutes. The antigen and saline are then well mixed and left in the water bath for 2 more minutes for ripening. The antigen should be kept in neutral bottles. One drop of active serum is mixed with 2 drops of diluted antigen and rotated for 30 seconds to ensure sufficient mixing on the slides which are then left at room temperature. The results are read with the naked eye after 30 minutes.

Mueller Ballung (Clotting) test : 2 ml. of antigen are mixed with 3 ml. of alkalised saline at 17°C. After 10 minutes 25 ml. of alkalised saline of 17°C are added to the

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mixture and the antigen is ready for use. Alkalised saline : Prepare a main solution of 3 g. sodium carbonate in 100 ml. of normal saline. 10 ml. of this solution are added to 990 ml. of normal saline. Four drops of inactivated sera are added first and 0·5 ml. of diluted antigen are subsequently added and mixed. The tubes are incubated for 10 minutes at 56°C in a waterbath and then left at room temperature. The results are read after 18-24 hours.

The sera were inactivated for 3 minutes at 60°C (Velaudapillai 1950) and the method of preparing Cardiolipin antigen as well as the test is described in the same paper.

The sera for Cardiolipin, Meinicke and Mueller Ballung tests are added by a capillary pipette graduated to deliver 0·025 ml. per drop. This is done by cutting the pippettes at the 56th hole of starret's gauge.

Results

Out of the total of 15,057 sera, an exact clinical diagnosis was obtainable for 2,560 specimens.

The serological results obtained are shown in table I.

TABLE I

	All sera (15057)		*Syphilitic sera* (*All stages*) (1211)		*Non-syphilitic sera* (1349)	
	Reactors		*Reactors*		*Reactors*	
	No.	%	*No.*	%	*No.*	%
KR	3853	25·6	759	62·7	52	3·8
CL	4396	29·2	819	67·6	73	5·4
MK	3720	24·7	718	59·3	44	3·3
MB	3855	25·6	705	58·2	55	4·1

Key : KR Kahn reaction
CL Cardiolipin (Kline) test
MK Meinicke (Kvittingen) test
MB Mueller Ballung (Clotting) test.

N.B.—Doubtful reactions are included as reactors.

Serologically the Cardiolipin test proved to be more sensitive, the other tests are closely related in their results. The Cardiolipin test is somewhat less specific in non-syphilitic sera, it gives, however the best agreement with the clinical diagnosis as regards syphilis. The Meinicke (Kvittingen) test gave the lowest percentage of positive results in non-syphilitic sera. The Mueller Ballung test is inferior to the Meinicke (Kvittingen) test.

To evaluate the above outlined results some references to the techniques are to be made. The Kvittingen test was developed in the continent and needs there a storage in a moist chamber for 25 minutes at 37°C, as room temperature and humidity are too low as to favour an immediate reaction to take place. In the tropics temperature is high enough to avoid the need of an incubator and in areas such as

Colombo, the humidity present in the air is high enough to permit the test to be performed without these equipment. The Kvittingen test was run parallel (*a*) at room temperature and room humidity, (*b*) wet chamber at 37°C. No difference of results were observed in this trial. The humidity was above 75 per cent., the temperature about 28°C. Further trials in hot, but dry areas are still to be performed to establish an average threshold of humidity permitting the test to be performed under room conditions. Further more the test is done on active sera, hence equipment is minimal, thus labelling the test suitable for massfield testings.

The Mueller-Ballung (clotting) test is of high sensitivity and specificity in temperate climates and is used as a confirmation test in doubtful cases. The test is not a quick reaction but needs about 18 hours at room temperature to form the clot in positive sera. As outlined above there is a mean difference of 8-10°C between the mean room temperatures in Europe and Ceylon (Mean temp. Vienna 9·15°, Colombo 26·94°C). This raised temperature influences the clot formation and tends to form unspecific clottings. These facts, as well as the prescribed low temperature (17°C) when preparing the antigen involve many factors of uncertainty which deprive this test in the tropics of his high value well established in temperate climates.

The comparison of sets of 2 tests each for agreement of their results are given in table II.

TABLE II

	All sera (15057) *Identical reactions*		*Syphilitic sera (all stages)* (1211) *Identical reactions*		*Non-syphilitic sera* (1349) *Identical reactions*	
	No.	%	*No.*	%	*No.*	%
KR : CL	13439	89·3	982	81·1	1279	94·8
KR : MK	13323	88·5	948	78·3	1299	96·3
KR : MB	13177	87·5	919	75·9	1298	96·3
CL : MK	13359	88·7	937	77·4	1289	95·5
CL : MB	12990	86·3	905	74·8	1275	94·5
MK : MB	13189	87·6	948	78·3	1294	95·9

Key—see Table I.

The best overall agreement was found for the set Kahn and Cardiolipin tests. The same combination proved to be the best in syphilitic sera, whereas in non-syphilitic sera combinations with Meinicke (Kvittingen) give better agreement with the clinical diagnosis.

Besides the Kahn and Cardiolipin combinations the other combinations give agreements to nearly the same degree. The Cardiolipin and Meinicke tests are done with ease, and as such these two combinations have an advantage over the other combinations.

Yaws was diagnosed for 20 sera, and 18 of them were positive in all 4 reactions. One was negative in the Mueller Ballung (Clotting) test only, the other in Kahn and Cardiolipin tests.

Leprosy was diagnosed for 151 sera, these patients had no clinical evidence or history of syphilis or yaws. The results of the 4 tests are shown in table III.

TABLE III

	Reactors	
	No.	%
KR	21	13·2
CL	19	12·6
MK	21	13·2
MB	23	15·2

Key—see Table I.

False positive results are given by all 4 tests. Cardiolipin test gives the lowest (12·6 per cent.) and Mueller Ballung (Clotting) test the highest (15·2 per cent.) rate of reactions.

Discussion

It will be observed that the Cardiolipin test is the most sensitive test and the other tests within acceptable limits are equally sensitive and specific. From the practical point of view Cardiolipin and Meinicke tests should be considered as tests of choice. They are easy to perform and in laboratories where 2 tests are done, it is suggested that Cardiolipin and Meinicke tests be performed. In laboratories where equipment is inadequate Meinicke test might be used, as the test is done with active sera at room temperature. The antigen could be prepared with a spirit lamp and a beaker of water. The work was done in Colombo, and for the present it is recommended that Meinicke test be given a trial in places where climatic conditions similar to Colombo exist.

Summary

The Kahn, Cardiolipin (Kline), Meinicke (Kvittingen), and Mueller Ballung (Clotting) tests are compared on 15,057 samples of routine serodiagnosis. Simplification of the Meinicke (Kvittingen) technique is described and this test is recommended as suitable for field conditions.

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