

THE CLINICAL VALUE OF THE COMBINED KAHN AND WASSERMANN TESTS IN THE TROPICS, WITH SPECIAL REFERENCE TO YAWS AND SYPHILIS.

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CONTENTS.

	PAGE
Introduction	215
The Author's observations	218
Summary	223
References	224

INTRODUCTION.

In tropical Africa the serological diagnosis of yaws and syphilis by means of the Wassermann reaction is notoriously beset with difficulties. The reason for these difficulties is within easy reach. Bigger (1921, 1921 *a*) pointed out that attempts to standardise the Wassermann reaction could never be completely successful, owing to the fact that each of the four reagents employed in the test is more or less variable. This variability becomes far more apparent when the test is undertaken in the tropics. The temperature affects adversely the stability of the reagents, and the precarious conditions attached to the keeping of stock animals interferes with the supply of constantly reliable sera.

A succession of failures directly attributable to local climatic conditions caused me to consider the choice of another test, less sensitive to the vicissitudes of a tropical climate, which would fulfil the requirements of the Wassermann reaction whilst still preserving its diagnostic reliability. The less complex the test and the fewer its reagents, the less liable is it to be affected by conditions of climate and temperature. The ideal test, therefore, would require to combine a minimum of complexity with a maximum of accuracy. At the time the number of tests claiming to equal the Wassermann reaction in value was large and steadily increasing. The Kahn, Sigma, Sachs-Georgi, Meinicke, Müller, Murata, Vernes tests—and these were not all—each had their advocates. Out of these collective methods it was difficult to make a choice, but the first-named appeared to have more supporters than any of the other flocculation tests.

Numerous observers in different countries have at various times reported on the use of the Kahn test performed in parallel with a complement fixation test. McLean (1927) testing 767 sera recorded a 57·6 per cent. agreement between the Kahn and Wassermann tests. Figueira and Trincao (1929) with 1076 sera showed an agreement of 88·1 per cent. and whilst considering the Kahn a more

sensitive test in treated cases, did not find it as diagnostic as the Wassermann. Davenport (1930) carried out 2070 tests by the Kahn technique, and states that in florid cases of secondary syphilis it gave 100 per cent. strongly positive results. He did not find, however, that the changes in treated cases ran parallel with the degree of treatment. Whilst attributing a high degree of sensitiveness and specificity to the test, he believes it to be most useful as a diagnostic procedure rather than a measure of therapeutic response. McIntyre and Gilman (1929) were unable to find sufficient evidence to warrant the supplanting of one test by the other, when comparing the Kahn and Kolmer techniques. They maintain that the clinician and his patient are best served by the performance of both tests. Cookson and Brown (1930) comparing 1000 sera by the Wassermann-Kahn combination found the Wassermann more sensitive even in treated cases, and believe that the Kahn should not supplant it, but that it is most valuable to do both tests. They stressed the simplicity of the Kahn where the Wassermann was not possible, as in the tropics.

Tropical workers have also reported on the Kahn and Wassermann in parallel tests. Butler (1923) with 192 sera and an agreement of 81 per cent., found the Kahn test more sensitive to treatment and apparently more sensitive to yaws than to syphilis. Later (1924) he confirmed his views on sensitiveness with 486 sera. This work was carried out in Tanganyika Territory. Kauntze (1926) in Kenya Colony did not find the Kahn test reliable, except as a rapid method. Pineda (1926) in the Phillipines obtained a 89.4 per cent. agreement in yaws cases. Greval (1929) in India, with 1000 of his cases, reported a 69 per cent. agreement between Kahn and Wassermann, and expressed the view that the Kahn test was neither as specific nor as sensitive as the Wassermann. The present writer (1928), in Tanganyika Territory, performed parallel Kahn and Wassermann tests on 86 sera and found an 82 per cent. agreement. Later (1929) with 332 sera he obtained an agreement of 82 per cent.

Reviewing the results of these different observers it is apparent that there is a considerable diversity of opinion regarding the various features of the Kahn test in its relation to the Wassermann reaction. Not only have discrepancies been recorded regarding its actual agreement with the Wassermann test, but opinions have been divided concerning its specificity, sensitiveness and relation to treatment. It will be observed that none of the tropical workers previously mentioned have attributed their inconclusive results directly to the influence of climate: indeed, such influences would be more likely to affect the Wassermann itself rather than the less complex Kahn reaction.

The explanation of anomalous results may be found in several factors other than those associated with climatic conditions. Of these, the personal element is an important feature. There is little doubt that flocculation tests, although credited with a reputation for simplicity, have earned that reputation more in their theory than in their practice. Flocculation tests are admittedly less complicated than complement-fixation tests, because of the smaller number of variable reagents used: but their technique does not because of that require

any less accuracy or skill in its performance. Another factor which may throw some light on the lack of correlation between the results of different workers is the nature of the nomenclature ordinarily employed in the Kahn method. Fine degrees of strong and weak positive reactions must be recorded in the purely scientific research of specificity and sensitiveness: but in general use a nomenclature involving the use of six different signs is more likely to produce discrepant results than one which is confined to three. For the purposes of clinical comparison, the simple "positive," "doubtful" and "negative" notation seems to be of more practical value. Finally, most comparisons regarding the utility of any test have been made in terms of the agreement existing between that test and another serological standard. In the cases under review, that standard has been the Wassermann reaction or one of its modifications.

Davenport (1930) states that "the true value of any test may best be established by comparison with the clinical observation." In the opinion of the writer, most value should be obtained in the use of two well-established tests interpreted in relation to each other and to the clinical data. This procedure should prove particularly useful in the tropics. Here, multiple diseases are frequently present in natives, and may in some way alter their serological state, so that the interpretation of the tests must be made with due regard to the combined clinical and serological observations.

In May, 1928, the League of Nations Health Organisation held a Laboratory Conference on the Serodiagnosis of Syphilis at Copenhagen. In an attempt to establish some standardisation of serodiagnostic methods, they invited distinguished serologists from different parts of the world to perform their various tests on a common batch of sera. From that conference a mass of instructive information has been derived. Full details of the findings may be obtained by referring to the League's *Report*. It might not be out of place, however, to touch briefly on the principal points arising out of the conference:

(1) Of the flocculation tests, the Kahn showed no non-specific reactions, and with the Müller test, gave considerably more positive reactions with syphilis cases—303 out of 495—than any other testing this number.

(2) The highest number of positive Bordet-Wassermann reactions in 495 syphilis cases showing no non-specific reaction was 208.

(3) The Kahn and Müller tests proved more delicate than any other.

(As Kahn has pointed out (1929) the Müller test lacked practicability, whilst the Kahn test fulfilled the four requirements of the conference—practicability, specificity, sensitiveness and clear-cut reactions.)

The conference suggested the following main resolutions:

(a) The best of the flocculation tests is equal in value to the best of the Bordet-Wassermann, but flocculation tests despite their apparent simplicity are very sensitive to differences in experimental conditions and must be in the hands of well trained serologists.

(b) The most reliable information for the clinician is derived from at least two methods.

(c) Serologists should check the accuracy of their results by frequent reference to clinical data.

(d) An uniform notation is desirable, that suggested being + to indicate results which in the view of the pathologist are given only by sera from cases of syphilis (and a few well-defined conditions); \pm to denote reactions which are neither negative nor positive; - to denote negative reactions.

(e) In the absence of a clear history or signs of syphilis, a positive reaction should not be accepted until a test of at least one more specimen has afforded the same result.

(f) The value of reports on tests of serum would be enhanced if clinicians would study closely the diagnostic and therapeutic implications of such reports.

Other suggestions were made which need not be mentioned here.

It will be observed that most of the causes of confusion existing in the results of different workers were touched upon in the resolutions of the conference, and that methods of avoiding such confusion were recommended.

THE AUTHOR'S OBSERVATIONS.

The present work has been carried out, and the results thereof interpreted in accordance with the resolutions put forward by the conference. The writer has not discovered in the literature any detailed record of a combination of the Kahn and Wassermann reactions performed in East Africa under these conditions and it is hoped that some account of the work may be of general interest.

The purposes of the investigation were the following:

(1) To examine the practicability of the Kahn test as a serodiagnostic method in the tropics.

(2) To compare its results with the results of the Wassermann reaction carried out in parallel and under similar conditions.

(3) To assess the value of the tests in combination and singly in relation to the clinical data.

The question as to whether either test was capable of demonstrating a serological difference between yaws and syphilis could not be undertaken satisfactorily. Most of the sera were from yaws cases, and in many instances no detailed information was received regarding the clinical types.

The Wassermann reaction employed was the No. IV method recommended by the Medical Research Committee, and differed only from the original in that it was carried out volumetrically with capillary pipettes.

The "Routine Diagnostic Test" described by Kahn (1928) was used, and his original description was closely followed.

In recording the first series of these tests (1929), the writer made use of Kahn's notation and the Medical Research Council's notation in the respective tests. Since the publication of the League of Nations' resolutions, the total number of tests has been re-interpreted by the nomenclature recommended by

the conference. This has served to alter materially the percentage of agreement between the two tests.

The notation used in the writer's previous reports on 332 tests was:

Kahn		Wassermann
+ + + +		
+ + +	corresponding to	+ +
+ +	" "	+
+ and ±	" "	±
-	" "	-

In the present report the results have been interpreted in both tests as +, ± and -. This correlates with the previous notation thus:

League of Nations		Kahn		Wassermann
+		+ + + +		+ +
		+ + +		+
		+ +		
±		+ or ±		±
-		-		-

500 sera were examined in parallel. The total results were as follows:

Kahn		Wassermann		
		+	±	-
+	191	187	3	1
±	34	4	16	14
-	275	1	2	272
	500	192	21	287

The actual correlation between the two tests was therefore 95 per cent. Of the 5 per cent. disagreements, 80 per cent. were due to the Kahn giving more positive and doubtful results than the Wassermann.

230 of the sera were from patients diagnosed as suffering from yaws and syphilis, 218 were from patients diagnosed as not suffering from either of these conditions, and of the remaining 52 sera no details were received.

As these investigations are primarily concerned with the clinical value of the tests, these 52 sera from unknown sources will, for the purposes of the investigations, be discarded. All the results discussed in the remainder of this report will therefore be regarded as applying to the 448 diagnosed cases only.

The total results from the clinical standpoint were:

Kahn		Wassermann		
		+	±	-
+	182	179	3	0
±	25	1	14	10
-	241	1	1	239
	448	181	18	249

The correlation is 96.4 per cent. The positive, doubtful and negative results by the tests singly and in combination is shown in the following table:

	+	±	-
Kahn and Wassermann reaction agreement	179	14	239
Kahn only	182	25	241
Wassermann reaction	181	18	249

More interest is perhaps attached to the results when stated in relation to the separate clinical diagnoses. In the following tables, each condition is considered separately:

184 sera from untreated cases of yaws and syphilis.

Kahn		Wassermann		
		+	±	-
+	156	154	2	0
±	14	1	5	8
-	14	0	0	14
184		155	7	22

The correlation is 94 per cent. The results by each method are stated below:

					+	±	-
Kahn and Wassermann reaction agreement					154	5	14
Kahn only	156	14	14
Wassermann reaction only					155	7	22

The Kahn showed the larger number of positives and doubtful reactions.

46 sera from cases of yaws and syphilis undergoing treatment.

Kahn		Wassermann		
		+	±	-
+	17	16	1	0
±	10	0	9	1
-	19	0	0	19
46		16	10	20

The correlation is 95.6 per cent. The following are the results by each method:

					+	±	-
Kahn and Wassermann reaction agreement					16	9	19
Kahn only	17	10	19
Wassermann reaction only					16	10	20

The Kahn gave slightly more positive reactions. The nature and amount of treatment given was not always specified, so that no figures can be given to illustrate the serological changes brought about by any individual forms of treatment.

218 sera from cases diagnosed as neither yaws nor syphilis.

Kahn		Wassermann		
		+	±	-
+	9	9	0	0
±	1	0	0	1
-	208	1	1	206
218		10	1	207

The correlation is 98.6 per cent. The individual findings are:

					+	±	-
Kahn and Wassermann reaction agreement					9	0	206
Kahn only	9	1	208
Wassermann reaction only					10	1	207

The Kahn gave more negative reactions.

SUMMARY OF RESULTS.

(A)	Yaws and syphilis untreated...	...	184 sera
(B)	Yaws and syphilis treated	...	46 „
(C)	Neither yaws nor syphilis	...	218 „

	Positive			Doubtful			Negative		
	Wassermann-Kahn	Kahn	Wassermann	Wassermann-Kahn	Kahn	Wassermann	Wassermann-Kahn	Kahn	Wassermann
(A)	154	156	155	5	14	7	14	14	22
(B)	16	17	16	9	10	10	19	19	20
(C)	9	9	10	0	1	1	206	208	207
	179	182	181	14	25	18	239	241	249

Results as percentages of the number of sera in each group.

(A)	83.1	84.7	84.2	2.7	7.6	3.8	7.6	7.6	11.9
(B)	34.7	36.9	34.7	19.5	21.7	21.7	41.3	41.3	43.4
(C)	4.1	4.1	4.5	0.0	0.4	0.4	94.4	95.4	94.9

Omitting the doubtful reactions, the results may be stated thus:

		% positive	% negative
(A)	Wassermann reaction and Kahn combined	83.1	7.6
	Kahn only	84.7	7.6
	Wassermann only	84.2	11.9
(B)	Wassermann reaction and Kahn combined	34.7	41.3
	Kahn only	36.9	41.3
	Wassermann only	34.7	43.4
(C)	Wassermann reaction and Kahn combined	4.1	94.4
	Kahn only	4.1	95.4
	Wassermann only	4.5	94.9

It will be observed that over 4 per cent. of sera from patients believed not to be suffering from yaws or syphilis gave positive reactions. Strictly, these should be regarded as false positives. Such would be the interpretation of these results if encountered during the course of an investigation of this nature in European practice, where non-syphilitic sera could be obtained with ease. The sera in this series were often sent in the course of ordinary routine and accompanied by a provisional diagnosis. The need of two tests in the tropics becomes more apparent when one realises that doubtful diagnoses are necessarily far from uncommon. Those who are familiar with medical practice in tropical Africa are aware of the difficulty which frequently arises in eliminating completely all suspicion of past or present syphilis and yaws in native patients. It is significant that in the nine cases under review a strongly positive reaction was actually given by both the Khan and the Wassermann tests. One would hesitate before indicting either test on a charge of non-specificity in the circumstances. If it is accepted that the diagnosis in these four cases was incorrect, then one may claim that the Kahn test gave no non-specific reaction. One positive reaction was given by the Wassermann alone on a serum believed not to be from a yaws or syphilis case.

The disagreements may be conveniently analysed together as follows:

	Kahn + Wasser- mann ±	Kahn + Wasser- mann -	Kahn ± Wasser- mann +	Kahn ± Wasser- mann -	Kahn - Wasser- mann +	Kahn - Wasser- mann ±
Yaws and syphilis (untreated)	2	0	1	8	0	0
Yaws and syphilis (treated)	1	0	0	1	0	0
Neither yaws nor syphilis	0	0	0	1	1	1
	3	0	1	10	1	1

The actual cases whose sera did not agree are of interest and are tabulated, together with their clinical and serological diagnoses in the following list:

Untreated cases.

	Kahn	Wassermann
(1) Primary syphilis—10 weeks ...	+	±
(2) Friedreich's ataxia... ..	+	±
(3) Secondary yaws	±	±
(4) Tertiary syphilis	±	-
(5) Yaws	±	-
(6) Primary syphilis	±	-
(7) Secondary syphilis... ..	±	-
(8) Crab yaws	±	-
(9) Seedy yaws... ..	±	-
(10) Crab yaws	±	-
(11) Tabes dorsalis	±	-

Treated cases.

(12) Gangosa—history of yaws ...	+	±
(13) Syphilis—previously + both tests...	±	-

Not yaws or syphilis.

(14) Leprosy	±	-
(15) Not diagnosed	-	+
(16) Hemiplegia—no history of syphilis	-	±

Thus it will be seen that over 80 per cent. of errors in agreement were due to the larger number of positive and doubtful reactions given by the Kahn test. Over 60 per cent. were attributable to ± reactions given by the same test in yaws and syphilis cases. The three Kahn + tests were all accompanied by a ± Wassermann reaction, and it is of interest to note that the clinical condition in each case was one in which a doubtful reaction would commonly be seen.

Kahn claims for his technique a sensitiveness to the effects of treatment which is not observed in other tests. This is due to the three-tube technique, and was actually observed frequently during the present work. The nomenclature adopted tends to mask this phenomenon.

It may be said from analysis of all these results that, in the series of tests under review, the Kahn test satisfied at least three of the four requirements demanded by the League of Nations' Conference—namely, practicability, sensitiveness and specificity. The fourth requirement—clear-cut reactions—did not, in my experience, appear to be satisfied as fully by the Kahn as by the

Wassermann test. This is no doubt a personal view which may alter with increasing practice.

The Kahn test proved its practicability for use in the tropics by the fact that its only special reagent—cholesterinised antigen—showed itself to be as stable as the Wassermann reagents, even when kept for two months at room temperature. Sensitiveness was shown by the large number of its positive reactions which agreed with the clinical findings. Specificity was manifest by the absence of any undoubted false positive result. If the results of the test are interpreted in strict accordance with the League of Nations' resolutions it should prove a reliable diagnostic agent, and even a measure of the progress of treatment, in the tropics and where the Wassermann test is not practicable. When both tests are available, however, it is most instructive to perform them in parallel, the one checking rather than supplanting, the other.

SUMMARY.

1. Sera from 500 patients, mostly natives of East Africa, were tested by the Kahn and Wassermann tests, under conditions described.
2. Clinical data were available in the case of 448 specimens. The results of tests upon 230 sera from cases believed to be yaws and syphilis and 218 cases believed not to be yaws and syphilis are discussed. In 46 of the 230 cases, treatment had previously been given.
3. In interpreting results, the resolutions of the League of Nations' Conference on the Serodiagnosis of Syphilis were strictly followed.
4. In the performance of the tests recognised standard methods were used.
5. The two tests correlated exactly in 96.4 per cent. of all cases.
6. The Kahn test was the more sensitive to yaws and syphilis.
7. The Wassermann test alone gave one non-specific reaction.
8. The reagents used in the Kahn test did not deteriorate when kept for two months at room temperature.
9. It is suggested that although the use of both tests is preferable when practicable, the Kahn test alone is a reliable diagnostic measure in the tropics.

I have to thank Dr J. O. Shircore, C.M.G., Director of Medical and Sanitary Services, Tanganyika Territory, who has given his permission for the publication of these notes.

My thanks are also due to the many Medical Officers who have responded to my request for specimens of sera for the tests, and particularly Drs Graham, Steel and Williamson, who have supplied the bulk of the specimens.

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(MS. received for publication 28. VIII. 1930.—Ed.)