

## THE APPLICATION OF THE SEROLOGICAL TESTS \* FOR SYPHILIS

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### INTRODUCTION

Some diseases, such as yaws and yellow fever, through some peculiarity in the method of transmission or of the intermediate insect host, appear to be rather strictly confined to hot climates, and are therefor properly called tropical diseases.

Other diseases, such as cholera, leprosy and plague, are today chiefly found in tropical climates, merely because defective sanitation in certain tropical countries permits the spread of these diseases; but they are not tropical diseases. They were formerly common in Europe and other cold climates, and leprosy still exists in Scandinavia and plague in Manchuria.

Still another type of diseases are so transmitted that they are common in any climate or country. The venereal and respiratory diseases belong in this category, and syphilis and tuberculosis are to be found wherever people congregate, and in every country constitute major problems for the sanitary authorities. For this reason, and because for a number of years I have been interested in the serological and the public health aspects of syphilis, I have taken the above subject for discussion.

It is not to be supposed that syphilis is more of a sanitary problem in Porto Rico than in other countries, but that the problem exists in your fair island, I suppose no one would deny. In 1914 I had the opportunity of making a Wasserman survey<sup>(1)</sup> of the Porto Rico regiment of infantry. Of 531 enlisted men so examined, 37.4 per cent had a completely positive Wassermann reaction, and this combined with the clinical history indicated an infection rate of 45 per cent. While this was by no means to be taken as a general rate for the community, it was fair evidence that the problem of syphilis existed at that time and unless Porto Rico is very different from the United States, that it still exists today though perhaps to a lesser extent than formerly.

Now I think that to-day practically all authorities agree not only

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that the most hopeful method of attacking this problem is the diagnosis and treatment of those who suffer from this disease, but that in the management of patients, we should soft-pedal the venereal origin of the disease. As a matter of fact, including the accidental cases which may be from five to ten per cent of the total number of infections, the congenital cases, and marital syphilis, about half the cases are innocent, and this fact greatly complicates both the diagnosis and the treatment of this disease.

In the serological tests, the Wassermann, and the Kahn, we have our most useful diagnostic aids, and by their means many cases of syphilis may be detected that would never have been suspected from the history and clinical examination. But when these tests are only requested in suspected cases, not only are the many unsuspected cases thereby missed but often patients are deeply insulted by the suspicion. Those hospitals and private physicians who make the taking of a specimen for this purpose an invariable routine of every physical examination are enabled to dodge both horns of this dilemma. The information so obtained is vastly more important and valuable than that obtained from a routine examination of the urine which has been practiced for so many years. It is therefore desirable that these tests should be made a routine part of the examination of any patients requiring medical attention.

Any tests that are to be so generally used should be thoroughly understood. Although the great value of these serological tests is universally admitted, and volumes have been written concerning their interpretation, there is as yet no absolute concensus of opinion as to their precise significance, and the further discussion of certain points is not entirely a work of supererogation.

1. Does a positive test, in the absence of all history of physical signs of the disease, mean the existence of syphilis (or yaws)?

The Second International Laboratory Conference on the serodiagnosis of syphilis, held in Copenhagen, May 20-June 4, 1928, at the invitation of the League of Nations, and attended by thirty-seven invited representatives from nineteen countries, answered this question in the following resolutions<sup>(2)</sup>.

1. That, in spite of increased sensitiveness which the various serodiagnostic methods have shown at the present conference, serological results may, notwithstanding the presence of a syphilitic infection, be negative in certain cases.

2. That a positive reaction in the absence of a clear history or of signs of syphilis should, if only to exclude all possibility of error,

never be accepted until a test of at least one more specimen has afforded the same result.

3. That except in the case of a few well defined pathological conditions, syphilis is indicated with a degree of probability which closely approaches certainty, when several tests performed according to different methods give a positive result.

Accepting these conclusions, in the Philippines, Porto Rico, and many other tropical countries, the tests must always be interpreted in view of the possibility of antecedent yaws, which is the one and only pathological condition known to give a positive reaction with much the same certainty as syphilis. In other conditions, including leprosy, when a proper technique is used, positive reactions occur only in a relatively small per cent of cases, not higher than the percentage of unsuspected syphilis or yaws in the community as a whole.

With these conclusions few will today differ. I must however disagree with those clinicians who maintain that the Wassermann reaction must be *verified* by the clinical examination, or that the Wassermann reaction should not be performed except in a syphilitic clinic where the results can be so verified.

Of course we cannot use these very useful tests as a mere rule of thumb, for there are fairly numerous cases of syphilis, chiefly tertiary, and old latent infections, that present negative Wassermann reactions. The clinician and the syphilographer find scope for their diagnostic acumen in these cases, which also serve as an incentive to the serologists to maintain and if possible increase the delicacy of these reactions. This has been measurably accomplished by the simultaneous use of the Kahn test.

No one should object to the conclusion of the League of Nations Conference<sup>(3)</sup> that Serologists should check the accuracy of their tests by regular and very frequent reference to clinical data in consultation with clinicians. No scientific serologist would be satisfied with anything less. But it is equally true that the serological reactions should be a check upon the clinical examination, a point that has been somewhat obscured by some syphilologists. Granting that a positive Wassermann is not infallible when properly performed it approaches infallibility much closer than the most careful clinical examination, and the best clinicians will miss more cases of syphilis without the Wassermann, than the Wassermann (employed upon both blood and spinal fluid) will miss without the clinician. A repeatedly positive Wassermann and Kahn in a case that is negative clinically, may be regarded as an evidence of the inadequacy of methods of

clinical examination just as truly as a negative reaction in a case of undoubted syphilis indicates the inadequacy of serological methods. The fact is therefore, that while the clinical results act as a check on the serological methods, these methods act equally as a check on the clinician.

II. What is the mechanism and relative value of the Wassermann and Kahn reactions? The mechanism of these tests is most complex and is not entirely known. Those who are interested in the structure and action of complement, haemolysin and antigen will find these details ably discussed by Zinsser<sup>(4)</sup> and Wells<sup>(5)</sup>. It is sufficient to state here that when the antigen and a positive serum (two colloid solutions) are mixed in the proper proportions, a fine precipitate occurs upon which the complement is absorbed, so that haemolysis does not occur upon the subsequent addition of the red cells and haemolysin. When the antigen and positive serum are mixed in somewhat different proportions in the Kahn test, the precipitate formed is more flocculent and is therefore readily seen and read directly, without the interposition of the haemolytic system. The mechanism of the two tests is therefore essentially the same, in that they both depend upon the fact that when two colloidal solutions whose micells bear opposite electrical charges are mixed, a precipitation of these particles will occur.

Many comparisons of the relative specificity and delicacy of the Wassermann and Kahn tests have been made by different serologists. There appears to be some evidence that as usually performed, the Kahn test is more sensitive<sup>(3)</sup>, especially in old treated cases<sup>(6)</sup>, but there is still some difference of opinion as to the relative sensitivity and specificity of the two tests<sup>(7)</sup>, but in general these two tests agree in at least ninety-five per cent of all cases. The fact that there is this large percentage of agreement is an indication that both are exceptionally reliable tests.

Some institutions have abandoned the Wassermann reaction in favor of the Kahn test, because of the supposed greater simplicity of the latter. The simplicity of the Kahn test has been somewhat exaggerated by some of its advocates. It is true that it does not require the use of the haemolytic system, but the manipulations must be performed with even greater attention to detail than in the case of the Wassermann, and the reading of the test requires considerable training and delicacy of perception. It is therefore a test that must be performed with great care and attention to technique, including

the pipetting of minute amounts accurately if it is to give reliable results, and for this reason should be confined to the hands of well trained serologists.

While under these circumstances it is a test of great reliability, there will always be some few cases that are positive by the Wassermann test and negative by the Kahn, and *vice versa*. In the opinion of the majority of serologists, both the Wassermann and the Kahn tests should be used. Each case will detect a few cases that the other will miss; but far more important, the testing of a serum by two independent and diverging methods which give agreement in ninety-five per cent of the cases, is an insurance against technical error which cannot be entirely excluded when a single test is performed, no matter how careful and competent the worker may be. When an absolute discrepancy occurs between the two tests, both should be repeated using the same serum used in the first test. Each test also serves as a check on the delicacy of the other, so that no great alteration in sensitivity of either test can occur without detection. Thus, the simultaneous use of both tests avoids the reporting of false positives, that *bete noir* of both serologists and syphilologists.

III. Is the reagin in syphilitic serum a true immune body, in which case it might persist after the infection had disappeared, or is it of such a nature that a positive reaction always indicates the existence of infecting agent?

In order to answer this question, we ought to know what makes a serum positive. The precise nature of this property of positive serum is not known, but there is good reason for believing that it is related to some property of the globuline, and especially the euglobulin which is frequently increased in the blood and spinal fluid of syphilitics. But there is no evidence that this globulin is a specific immune substance, for the globulins of normal sera may be so treated as to change a negative serum to a positive. It seems more probable that as the result of the syphilitic infection, the globulins of the serum undergo a physico-chemical change as the result of which they acquire the property of precipitating certain colloid solutions which is not possessed by normal globulins. This physico-chemical change seems to be of such a nature that the electric charge on the micelles of the globulin is altered, a theory that would account for the precipitation not only of the lipoid colloids in the antigen, but also for the well-known precipitation of colloidal gold solutions by the spinal fluids of neuro-syphilitics. If, therefore, we call this characteristic of the positive serum, "reagin", it must be understood that the term

is used merely as a matter of convenience, and not as suggesting the existence of a specific substance.

This reagin does not invariably run parallel with the spirochaete invasion, though it generally does. The reaction begins very generally at the time of appearance of the chancre, and is almost invariably positive at the stage of general invasion of the spirochaetes characterized by the general eruption. Nevertheless the strongest reactions are not found at this time, but in the late stages of certain improperly treated or neglected cases, in which there may be few or no symptoms, and presumably few spirochaetes. This condition is typically exemplified by those Wassermann-fast cases who have no apparent signs of the disease. The reagin, therefore, can hardly be derived directly from the spirochaetes, but from the tissues as the result of spirochaete activity; and the intensity of the reaction in a given case depends not only on the degree of activity of the spirochaetes, but also upon the peculiar reactivity of the particular patient.

But all true immune substances, antitoxins, agglutinins, etc., are presumably produced in this same way by the body cells that have been stimulated to this production by the injection of the specific antigen, and in this sense, all immunity is cellular.

Why may not the syphilitic reagin be an immune substance in the same way?

There are several reasons for the belief that this reagin is not a true immune body. In the first place it has been a generally accepted belief that there is no immunity to syphilis, but merely a resistance to a new infection so long as the first infection is present; and that as soon as the patient is cured, he can contract the disease again. It is true that Chesney<sup>(8)</sup> and others have advanced arguments against this view, and have criticised the experimental work of Neisser upon which this view was originally based. Chesney and Kemp<sup>(8)</sup>, page 35) found that while rabbits, which were originally inoculated in the testis and treated late in the course of the disease, were almost uniformly refractory to a second intratesticular inoculation, other syphilitic rabbits similarly treated and re-inoculated by a different method (deposition of virus upon a granulating wound on the back) were in fifty per cent of the cases susceptible to a second infection. Brown and Pearce<sup>(9)</sup> had shown several years earlier that super-infection was possible in experimental syphilis of the rabbit.

While this work indicates that re-infection or super-infection is

possible, at least in rabbits, without a cure of the original infection, and to this extent must modify the older opinion, it is very strong evidence for the view that there is no true immunity to syphilis. The more probable explanation of the partial or temporary resistance to infection is that eventually the *Treponema* reaches a state of equilibrium with its host, as a result of which the body no longer reacts in the characteristic way (the production of a chancre and secondaries) on re-infection. There is much in favor of this view.

It is well known that the *T. pallidum* is very early and widely distributed in the body, and that it remains for many years in places inaccessible to treatment, as in the blood vessels, brain and cord. Systematic and careful search for this organism, such as that conducted by Warthin<sup>(10)</sup>; has resulted in the demonstration of its presence in practically all cases of syphilis, even in old treated cases in whom the infection was latent, and when there was no suspicion of syphilis at the time of death. Warthin said, "I have never seen at necropsy a case of perfectly healed syphilis." Search, often prolonged, always reveals active latent lesion in aorta, heart or other organ. There is no evidence pathologically that the case of syphilis ever becomes wholly free of spirochaetes. This well known continued presence of *Treponema pallidum*, and its ability to adapt itself to its host, has led to the universal conclusion that *therapia sterilizans magna* is a failure, that few syphilitics are completely cured, and then, only when treated very early in the course of the disease. It is highly significant that it is only such cases in man that become susceptible to re-infection, with the production of a new chancre and secondaries.

Chesney argues that if resistance in syphilis is due to the persistence of foci of infection, the immunizing factor in syphilis is quite different from that observed in most other infections. But there are many infections that act similarly. There is no immunity to certain filterable virus diseases (hog cholera) without a living virus, and the same thing is true for several protozoal infections. In malaria the resistance to infection is now recognized to be due to the continued presence of the infection. The host has become so adapted to the continued presence of the parasites that no symptoms of the disease are produced, and the host continues to live in a malarial country without malarial attacks except when his resistance is lowered by chilling or intercurrent infection. Similarly, the resistance of birds to proteosoma has been demonstrated to be due to the continued presence of the organism.

A second reason for believing that there is no immunity in syphilis is the failure of the many attempts that have been made to produce active immunity to syphilitic infection either in man or the lower animals. Utilization of derivatives of syphilitic tissues, cultures of treponemes, syphilitic virus both living and dead, or products of the latter have been uniformly unsuccessful, providing that the disease itself was not produced. Attempts to confer passive immunity to syphilis by transfer of serum from immune persons or animals have likewise been fruitless.

Similarly, there has been no satisfactory demonstration of true immune antibodies against *T. pallidum*, in spite of many efforts to do so. The few positive results reported are open to the serious criticism that it was found that the agglutinins or other bodies were effective against *T. pallidum* in culture, but not against the organism from living sources. This suggests that these organisms in culture were not *T. pallidum*, a supposition rendered the more probable by the paper of Kast and Kolmer<sup>(11)</sup> in which these authors confess their inability to cultivate *T. pallidum* by any methods so far used, and discredit the cultural organisms previously obtained by many other investigators. Among this considerable amount of experimental work on possible syphilitic immune bodies may be mentioned that of Kolmer and Rule<sup>(12)</sup>, who found that syphilitic sera from patients with a positive Wassermann were no more spirochaeticidal than normal sera, and that sera from such syphilitics were without prophylactic activity when injected into rabbits that had been inoculated with *T. pallidum*. Thus no part of the acquired resistance to syphilis is humoral. But the reagin that causes the positive Wassermann reaction was in these sera, and consequently cannot be an immune body.

Thirdly and finally, similar complement fixation reactions have been developed for a series of different infections that obviously confer no immunity. Thus Craig<sup>(13)</sup> has developed a practical complement fixation reaction for amoebiasis, by means of which he has succeeded in detecting a number of cases of abscess of the liver that were previously unsuspected. Fairley<sup>(14)</sup> has perfected a similar reaction in hydatid disease and also in schistosomiasis. The former reaction is so delicate that it is stated that few cases of hydatid disease are now missed in Australia where the disease is common, whereas formerly the diagnosis was more or less fortuitous. These complement fixation reactions have therefore proved their value, and are quite specific for each infection, yet no one would suggest that

amoebiasis or hydatids confer immunity or even resistance to further infection, and the serum from such cases that causes the complement fixation when mixed with the appropriate antigen (cultural amoebae, fluid from hydatid cysts, and snails containing schistosome embryos) cannot owe this property to immune bodies. But still more significant, complement fixation has been in use for many years as a most delicate and accurate method of diagnosis for dourine or the infection of horses with *Trypanosoma equiperdum*, as well as for surra caused by *T. evansi*. These infections not only confer no immunity, but are progressive and uniformly fatal.

This question has been discussed at some length for it is of more than theoretic interest. If the reagin were a true immune body, it might persist for a variable period after all treponemata were destroyed, and therefore could not be used as an accurate guide to treatment. Conversely, if not a true immune body and if it is produced solely as the result of treponemata on the body, this property of the serum will disappear when all the organisms that provoked it are destroyed.

Lord<sup>(15)</sup> has made an attempt to verify the truth of the latter hypothesis by a study of Wassermann-fast cases. The criterion of Wassermann-fastness was one year of regular and active treatment with persistently positive tests. Of 900 cases of syphilis at the Johns Hopkins Hospitals between Oct. 1918 and Jan. 1927 who received such treatment, 118 were Wassermann-fast. In forty of these patients, the spinal fluid showed abnormalities characteristic of syphilis, other than the Wassermann; sixteen others developed clinical recurrence after treatment was withdrawn, so that in 56 or 47.5 per cent of the entire group it was possible to prove active syphilitic disease. In 58 more or 49 per cent serious damage due to the *Treponema pallidum* was demonstrated, although the activity of the disease could not be proven beyond a doubt. On the other hand, there were no cases of primary or uncomplicated secondary syphilis whose Wassermann reactions remained positive at the end of one year of regular and active treatment. Lord interpreted these findings to mean that a fixed positive blood Wassermann reaction, persisting in spite of regular and active treatment, means continued activity of the syphilitic process.

Theoretical reasoning, experimental methods, and practical experience, therefore, all give concordant results to the effect that the substance that causes the positive Wassermann is not a true immune body, but that its presence indicates continued spirochaetal

activity and therefore continued infection. The Wassermann reaction may therefore be used as a true guide to treatment. When to this we add that the Wassermann reaction is one of the first symptoms of the disease to appear, and one of the last to disappear after treatment is instituted, it is a fair deduction that in this reaction we have the best single guide to treatment. This is not to be understood to mean that we are to be moved by no other consideration, for the wise physician will always consider the patient, and will not lay himself open to the charge that he is treating the disease rather than the patient.

IV. *The intensity of a positive Wassermann as a guide to treatment.* This phase of the subject while not new, has been neglected by many serologists and clinicians, who are often satisfied with a report of complete fixation of four plus. The strength of these reactions actually varies within very wide limits, and when determined affords very valuable information as to the intensity and length of treatment required by the individual case, and as to the progress of the case while under treatment. It is the only method of obtaining information as to the serological condition of the Wassermann-fast cases.

Various methods have been used to determine the intensity of these positive reactions including the use of varying amounts of complement and antigen; but the best method is to titrate the serum, that is, perform the test with gradually decreasing amounts of the serum to be tested. Several different methods have been devised for performing and reporting such results. The one most used in the United States at present is that of Kolmer<sup>(16)</sup> who sets up five tubes containing varying amounts of serum as follows:

Tube	C. C.	Result
1.....	0.1.....	++++
2.....	0.05.....	++++
3.....	0.025.....	++++
4.....	0.005.....	++++
5.....	0.0025.....	.....

and this reaction would be recorded as 4431.

The objection to this method consists in the fact that the amounts of serum are not varied uniformly thereby reducing the delicacy of the quantitative measurement, and the further fact this method of reading the result is a formula requiring some effort and training in interpretation, and is not readily translated into numerical units.

For a number of years at the Army Medical School<sup>(17)</sup>, I made a practice of titrating all sera that were completely positive in 0.1 c.c., the amount used routinely, and with each succeeding tube containing just half as much serum as the preceding one. Assuming that the positive reaction in 0.1 c.c. is unity, a positive reaction in 0.05 c.c. is just twice as strong and may be represented as 2, and the reaction may be reported in Wassermann units as follows:

Tube	1.	2.	3.	4.	5.	6.	7.	8.
Serum .....	0.1	0.05	0.025	0.025	0.0082	0.0031	0.0015	0.0007
Units .....	1	2	4	8	16	32	64	128

Of 281 such positive sera so titrated, and in which I was able to obtain a history of the cases, the results were as follows:

Units	Cases	Per cent
1.....	65.....	23.1
2.....	89.....	24.2
4.....	66.....	23.8
8.....	65.....	23.1
16.....	8.....	2.8
32.....	4.....	1.4
64.....	3.....	1.0
128.....	1.....	0.8

Wassermann units as described here are not in general use but are of obvious utility. Indeed the chief merit of the Vernes flocculation test so much used in France and to a lesser extent in England, depends upon the fact that it is read in quantitative units which may be charted so that the progress of a case may be followed with all the definiteness of a mathematical curve. The comparative results of the ordinary routine Wassermann and Vernes tests in a case of treated secondary syphilis have been reported as follows:

Date	Wassermann	Vernes
March 13 .....	++++	148
March 20 .....	++++	135
April 2.....	++++	102
April 12.....	++++	110
April 20.....	++++	83
April 27.....	++++	42
May 4.....	+++	17
May 13.....	++	10
May 18.....	+	4
May 25.....	+	4
June 1.....	+	3

Thus, although the results of the tests at the serological confer-

ence at Copenhagen<sup>(3)</sup> indicated that the Vernes test was not so specific or delicate as the best of the Wassermann reactions or the Kahn test, there can be no doubt that as thus interpreted in numerical units, it gives more information as to the actual progress of the case.

Kahn<sup>(18)</sup> has also devised a method of reading his test quantitatively in units. When 0.15 c.c. of serum in undiluted form, mixed with 0.01 c.c. of the special antigen dilution (standard technique) results in a definite precipitation, it is interpreted as four unit reaction. By preparing varying dilutions of the serum with salt solution, the number of reacting units is computed by multiplying 4 by the dilution. Thus if 1:5 represents the highest dilution of serum capable of giving definite precipitation, the serum contains  $4 \times 5$  or 20 units. If 1:50 represents the highest dilution in which a serum gives definite precipitation, the serum contains  $4 \times 50$  or 200 units. According to Kahn's interpretation, sera giving anything from four plus to two plus reactions are to be considered definite precipitation in this quantitative method. Plus or doubtful reactions are considered negative.

If Wassermann units are to be used as a definite serological measure, it seems necessary to correlate them with the Kahn units since the latter are already in general use. It is evident that if reports are received to the effect that a given patient has four Wassermann units according to my system and forty Kahn units, the clinician will be in a quandary as to the precise significance of the report. This also would impute to the Kahn test a delicacy approximately ten times as great as the Wassermann, a superiority that does not exist even though it may appear, as Kahn claims, that the Kahn test is somewhat the more delicate of the two in a long series of cases.

The reason for this discrepancy is to be found in the fact that Kahn used a partial reaction (two plus) and a larger amount of serum (0.15 c.c.) in determining his units, while only complete fixation (four plus) and 0.1 c.c. serum was used in my scheme for the determination of the Wassermann unit.

I therefore suggested to Major Schwartz, who is now performing the serological work at the Army Medical School, that he make simultaneous parallel titrations of a number of positive sera in both the Wassermann and Kahn tests. In both tests the designation of units is purely arbitrary. Kahn arbitrarily assigns four units to definite precipitation in the routine test. It was found that if we

similarly assign four units to complete fixation (four plus) in the Wassermann test as performed by the Army technique when the amount of serum used in the usual 0.1 c.c., and in the further titration multiplying by the dilution, essential agreement was reached between the number of Wassermann units and Kahn units in each serum. The actual dilutions of the two tests, and their interpretation in units was as follows:

Dilution.....	WASSERMANN							KAHN						
	1/2.5	1/5	1/10	1/20	1/40	1/80	1/160	1/2.5	1/5	1/10	1/20	1/40	1/80	1/160
Units.....	10	20	40	80	160	320	640	10	20	40	80	160	320	640

This work has not yet been completed, but will eventually be published. The results in the first 45 parallel titrations are indicated in the following graph.

KAHN

Units	4	10	20	40	80	160	320	640
4.....	6	1						
10.....	2	3	1					
20.....			6	4				
40.....			6	9	2			
80.....				1	1			
160.....					2			
320.....							1	

..... Curve of absolute agreement.

From this it will be seen that there is a very close agreement of the results of the two tests as performed at the Army Medical School. That in some cases the Wassermann gives a higher number of units than the Kahn, and *vice versa*, but that the discrepancies have so far involved differences only in the reading of one tube or dilution. Since in this work each succeeding tube contains only half as much serum as the preceding, it seems probable that by using many more tubes and diminishing the amount of serum used in the two tests by tenths instead of halves, a still closer approximation of the results would be attained. This involves altogether too much labor, and too much serum to be practicable for routine work.

At the same time it is desirable to avoid the reporting of the two quantitative tests sperarately. Since there is such close agreement in the results of the two tests, and since both tests are highly specific, especially in these dilutions where by no possibility can there be any

false positives, the plan is advocated of averaging the results of the two tests and reporting the results in syphilitic units.

According to this system, if the Wassermann shows four units and the Kahn ten units, this would be interpreted and reported as seven syphilitic units. Eighty Wassermann units and forty Kahn units as sixty syphilitic units, etc.

This suggestion has the merit of combining the results of the Wasserman and Kahn tests, expressing this combined result in a strictly quantitative manner as syphilitic units, which will be more specific and delicate than the units of either test used separately.

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