

## A NEW AND SIMPLE METHOD FOR THE SERUM DIAGNOSIS OF SYPHILIS.<sup>1</sup>

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Since the important discovery by Wassermann of serodiagnosis of syphilis, an enormous amount of work has been done, and to-day its value as a diagnostic test is beyond dispute. The complexity of the original method and the requirement for a thorough knowledge of hæmolysis and skill in serological technique have, however, been a great obstacle to its introduction into general practice. I am convinced that, unless a great simplification of the method of the Wassermann test can be devised, it will never attain the usefulness which it really deserves. Many workers have attempted to simplify the method, but thus far, according to my view, without success.<sup>2</sup>

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<sup>2</sup>Judging from my own observations which extended over 100 cases, Bauer's method (*Deut. med. Woch.*, 1908, xxxiv, 698) is not only unreliable on account of the inconstant content in natural anti-sheep amboceptor in human serum, but it is also inconvenient, because it requires washed sheep's blood corpuscles and the special apparatus of a laboratory. The method of Detre and v. Brezovsky (*Wien. klin. Woch.*, 1908, xxi, 1700-1743) appears about equal in reliability to the original, but in spite of its ingenious technique the use of washed horse corpuscles interferes with its usefulness outside of a laboratory.

A so-called simple method of serum diagnosis of syphilis recently published by Tschernogubow (*Berl. klin. Woch.*, 1908, xlv, 2107) is of a different order since he states that the complement contained in the blood of patients is sufficient in quantity to reactivate anti-human amboceptor and cause hæmolysis of the same blood. No mention is made of the source and strength of the anti-human amboceptor referred to. Tschernogubow's method relies upon an unknown quantity of complement which varies in different specimens of human blood. As the complement and erythrocytes deteriorate rapidly, his method is inapplicable to old specimens. There is also no direct way of testing the inhibitory action of the antigen alone. Anti-human amboceptor derived from immunized rabbits is reactivable only by large amounts of human serum, which renders the method inconvenient. Finally the idea of Hecht (*Wien. klin. Woch.*,

All the methods so far proposed are indeed suited only for the fully equipped laboratory possessing centrifuge, thermostat, water-bath, etc.

I have, I believe, been successful in overcoming the difficulties of the situation by perfecting a new and simple method of syphilis serodiagnosis. This method offers definite advantage in point of delicacy and simplicity for the experimental serologist; and the reagents to be employed can be prepared for and put within reach of the practitioner so that the test may be made by any physician who is in the habit of making clinical laboratory tests, such as the Widal test, hæmoglobin estimation, etc.

#### PRINCIPLES OF THE METHOD.

The new method is based upon the same principle as Wassermann's and utilizes the Bordet-Gengou phenomenon of complement-fixation to determine the presence of the syphilis antibody in a given specimen of blood serum or cerebrospinal fluid. It differs, however, from the Wassermann method in using an anti-human hæmolytic system instead of an anti-sheep hæmolytic system. This difference may at first seem of trifling importance but it really is very significant since it affects the accuracy, ease and reliability with which the test can be applied. Wassermann's original method, it may be added for sake of clarity, is subject to an error arising from the presence in human serum of a varying amount of natural amboceptor capable of being reactivated by guinea-pig's complement. Many specimens of human serum contain in 0.1 c.c., which is the quantity usually employed in a test for complement fixation, as many as twenty units of anti-sheep amboceptor, while some specimens are entirely devoid of this amboceptor. I found by actual experiment that four units, but not two units, of the natural anti-sheep amboceptor prevent entirely the detection of one unit of syphilis antibody. When eight units of natural anti-sheep amboceptor and two units of syphilis antibody are brought together, 1908, xxi, 1742) that the native hæmolytic property of human serum alone may be used as an hæmolytic system in combination with washed sheep's corpuscles is impracticable, as the amount of human complement and amboceptor is so variable as to make the test unreliable without a special standardization of such serum.

the test remains completely negative. In other words, by the method of Wassermann the presence of syphilis antibody may be missed although in the absence of the natural anti-sheep amboceptor it would be readily detectable. This defect in the complement fixation system is not only inherent to the Wassermann method but also to all those which employ foreign blood corpuscles for which human serum contains natural hæmolytic amboceptors capable of being reactivated by the complement employed in the test.

I believe that the method which I have worked out eliminates completely this source of error, because human blood corpuscles are used as the hæmolytic indicator in combination with human serum, so that as no foreign natural hæmolytic amboceptor is accidentally present, its effects are excluded. The present method of the complement fixation test is carried out under conditions of definite and uniform sensitization of the blood corpuscles, thus enabling one to detect the presence of even a fractional part of one unit of syphilis antibody in a given specimen. It is impossible to miss one or more units of the antibody, which happens not infrequently with the Wassermann and similar methods.

#### DESCRIPTION OF THE METHOD.

In order to carry out the present method of diagnosis it is needless to say that the serum of the patient under question must be obtained. The patient's serum is then subjected to the usual complement fixation test with the reagent to be described immediately below and according to the technique specially adapted for the present method. I have developed so far two different ways of applying the test. In one the reagents are employed in liquid form and in the other, the reagents are used in a dried state on filter paper. The first way of making the test is adapted to general biological laboratories, and the second is adapted especially for the clinical laboratory. The descriptions which follow immediately refer to the laboratory method, but they do not differ essentially from those for the clinical laboratory except that the reagents employed are in the two different forms mentioned.

## REAGENTS REQUIRED FOR THE METHOD.

The following reagents are required for making a test by the present method:

1. *Anti-human hæmolytic amboceptor* prepared in rabbits by injecting them five or six times into the peritoneal cavity with increasing doses (up to 20 c.c.) of washed human blood corpuscles, allowing five days interval between each injection. The serum is collected from the immunized animal eight or nine days after the last injection. The titre must be stronger than 0.01 c.c. for complete hæmolysis.<sup>3</sup> I now use a serum of 0.001 c.c. titre.

2. *Complement*. Fresh guinea-pig serum.

3. *Antigen*. Alcoholic extract of organs or crude preparations of lecithin. To prepare the alcoholic extract of organs one part of mashed tissue (liver, kidney) is extracted with ten parts of absolute alcohol for several days at 37° C., filtered through paper and the filtrate concentrated to about one-third of its volume, and the fluid preserved. To prepare the lecithin solution for antigen, 0.3 gram is dissolved in 50 c.c. of absolute alcohol and then shaken with 50 c.c. of physiological salt solution and filtered. The filtrate must be clear.

4. *Suspension of human blood corpuscles*. This is prepared by mixing one drop of the blood of a normal person<sup>4</sup> with 4 c.c. of physiological salt solution.

5. *The serum to be tested*. About ten drops of blood from a patient are collected in a small test tube. The clear serum separated from the clot is employed for the test.

## METHOD OF MAKING THE TEST.

1. Take six clean test tubes (size 10 cm. x 1 cm.). In the first two of these, place one drop from a capillary pipette (or tube if necessary) of the patient's serum which is to be tested. In each of the second two tubes (which are to serve as controls) put one drop of the serum of a syphilitic case known to give the positive

<sup>3</sup> Complete hæmolysis of 1 c.c. of human blood suspension ( $\frac{1}{4}$  drop) in the presence of 0.025 c.c. of guinea-pig's fresh serum.

<sup>4</sup> The suspension can also be prepared with the patient's blood, but it must be used only in combination with the serum of the same patient.

reaction. In each of the third pair of tubes (also controls) put one drop of the serum of a normal person. Now to each of the six tubes add 1 c.c. of the suspension of human blood corpuscles and 0.04 c.c. of fresh guinea-pig serum as complement. Lastly into one of each of the three foregoing pairs of tubes put one drop of the antigen solution from a capillary pipette. The second tube of each pair receives no antigen.

2. After being well mixed by shaking, the six tubes are incubated at 37° C. for one hour.

3. At the end of the incubation, add two units of anti-human amboceptor to each tube and mix well by shaking. Incubate the tubes for two hours longer at 37° C. Read the reaction from time to time for the next ten to twelve hours during which the tubes are kept at room temperature.

The complete test is shown in the following chart.

CHART I.

	Test for Diagnosis.	1st Control Set.* Test with a Known Syphilitic Serum (Positive Reaction).	2d Control Set.† Test with a Normal Serum (Negative Reaction).
Control tube without antigen for each test.	a. Patient's serum. ○ b. Human blood suspension. c. Complement.	a'. Positive luetic serum. ○ b. Human blood suspension. c. Complement.	a''. Normal serum. ○ b. Human blood suspension. c. Complement.
Determinative tube containing antigen.	a. } ditto. ○ b. } c. } d. Antigen.	a'. } ditto. ○ b. } c. } d. Antigen.	a''. } ditto. ○ b. } c. } d. Antigen.
Incubation at 37° C. for 1 hour.			
Anti-human hæmolytic amboceptor to all tubes.			
Incubation at 37° C. for two hours longer, then at room temp.			

\* When many specimens of syphilitic sera are subjected to simultaneous tests this set of controls is scarcely necessary, as we usually find a good positive serum among them.

† This control set may be made without the addition of any human serum.

It is necessary to begin the test with one drop of the patient's serum. If the reaction is negative the test should be repeated with two drops. Sera are not infrequently met with, however, in which two drops are inhibitory without the presence of antigen. On this

account it is undesirable to use two drops as routine. Usually one drop reveals the presence of the specific antibody if it is present at all, and further test becomes almost unnecessary.

#### READING OF THE REACTION.

A negative reaction is indicated by complete hæmolysis in both tubes irrespective of the presence of the antigen. On the other hand, a positive reaction is shown by complete absence or partial inhibition of hæmolysis in the tube containing the antigen and complete hæmolysis in the tube without the antigen.

Complete hæmolysis in the negative control takes place usually within one hour or sometimes earlier. When the reaction is strongly positive, as it should be with the positive control set, the tube with the antigen remains absolutely free from any trace of hæmolysis even after a few days' standing at room temperature. In such a case the erythrocytes are strongly agglutinated and remain adherent to the bottom of the test-tube, leaving above a perfectly clear medium. In moderately positive cases there is a trace of hæmolysis, while the varying degree of positive reaction can readily be measured by the amount of the hæmoglobin liberated in the medium and especially by the amount of intact red corpuscles remaining in the fibrin shreds or whitish stroma masses. It should be mentioned that the tubes containing the antigen undergo hæmolysis somewhat more slowly than those without antigen, but complete hæmolysis must occur in both tubes of the negative control set, before the reaction of the other sets of tests is read. It is also necessary to note that every tube without antigen, irrespective of the nature of the specimen of blood serum therein contained, must undergo complete, or at least almost, complete hæmolysis before the final reading of reaction is made. In cases in which tubes without antigen do not undergo hæmolysis the test must be repeated with a smaller quantity of the human serum in order to obtain a definite result.

#### SIMPLIFIED METHOD FOR THE CLINICAL LABORATORY.

The method so far described is much simpler than the original test of Wassermann and can be quickly and easily carried out in any

laboratory where experimental work with serum is constantly being conducted. I have, moreover, been able so to modify the methods of handling the various factors in the test that it should be possible to carry it out with accuracy and dispatch in any clinical laboratory. These further modifications consist essentially in drying the reagents on paper and standardizing them. In this form they can be prepared on a large scale by commercial biological laboratories under supervision of a competent serologist, and placed on the market within ready reach of physicians. My experiments have shown that it is possible to do this with great accuracy and that the preparations so made are stable when kept under ordinary conditions. In the following paragraphs I will describe the method of preparing the filter paper reagents and the mode of employing them for the test.

*Anti-human amboceptor slips.* The serum of immune rabbits is taken up with filter paper and quickly dried (a few hours) by a current of air at low temperature (below 20° C.). If the immune serum is not very strong—the titre 0.01 c.c. for example—the serum should first be concentrated to one-third of its volume by means of a current of air before the paper is impregnated. This is necessary in order to secure a slip of small dimension for convenient use.) After complete desiccation, the impregnated paper is cut into pieces of equal dimensions, each one being of such a size as to contain two units of the amboceptor.

*Complement slips.* A rather thick filter paper is impregnated with fresh guinea-pig serum, similarly dried and cut into pieces of equal size. The activity of the complement slip must be titrated and compared with the fresh complement. The activity of the slip-complement may vary somewhat with every preparation, but one slip should possess such an activity as to correspond with that of 0.04 c.c. of the fresh guinea-pig serum.

*Antigen slips.* A crude preparation of lecithin of previously known antigenic value is dissolved in ether and similarly taken up into filter paper. It is also easy to impregnate the paper with the alcoholic organ extracts (such as from the liver of a congenitally syphilitic foetus), but a preliminary concentration of the extract by means of a current of air is necessary in this case. After impreg-

nation and complete desiccation, the impregnated paper is cut into equal parts, each being of such a size as to contain enough antigen for one tube.

These three reagents, the anti-human hæmolytic amboceptor, guinea-pig complement and antigen, in the impregnated filter papers, can be kept indefinitely at room temperature in a dry place. I have adopted as the dimension of each slip the size of about 5 mm. by 5 mm. It is understood that preliminary tests should always be made with the slips to prove activity and strength.

In employing the filter paper slips, they are dropped by means of forceps into the test tubes already containing the human blood suspension and patient's serum, in the order and at the intervals already stated for the respective reagents (complement, antigen, amboceptor) in the description for the method of making the test (Chart I). It is necessary to shake the tubes a few times at intervals in order to insure proper solution of the reagents. The incubation may be carried out perfectly well in the vest pocket if a thermostat is not at hand. Confusion is to be avoided by carefully labelling the various tubes. Each of the pairs of tubes making up, respectively, the test, and the two control sets, may conveniently be held together by a rubber band. There can be no confusion within one pair of tests since one of the two tubes contains one more slip (antigen) than the other.

I should advise any one contemplating the use of the method to make a few preliminary trials comparing cases definitely syphilitic with normal individuals before undertaking the diagnosis of cases of unknown nature. This would also have the advantage of accustoming the physician to the order of the procedures and the reactions and serves as a test of the reliability of the reagents.

#### RESULTS.

In all, four hundred and sixty-five specimens of serum have been examined by the method above outlined. One hundred and fifteen of these were examined at the same time by the original method of Wassermann. The results are tabulated as follows:



TABLE I.<sup>5</sup>

Amount of Treatment. Method.*	O		Slight.				Moderate.		Fairly Well.				Weil.			
	W	N	W	N	W	N	W	N	W	N	W	N	W	N		
Reaction.	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
Primary Lues.	4	2	6	0	1	0	1	0								
Secondary Lues.																
Manifest.	18	2	20	0	4	0	4	0	1	2	3	0				
Latent.	1	0	1	0	0	1	1	0	4	2	6	0	1	1	1	1
Tertiary Lues.																
Manifest.	3	0	3	0	4	0	4	0	8	0	8	0			2	1
Latent.									5	2	6	1	1	1	1	1
Suspects.	16	12	21	7											5	4
Non-syphilitic.	0	5	0	5											7	2

\* W = Wassermann's test; N = the present test.

Summarized the results are as follows:

Of 7 cases of primary syphilis the Wassermann test was positive in 5, the present test in all.

Of 27 cases of manifest secondary syphilis the Wassermann test was positive in 23, the present method in all 27.

Of 12 cases of latent secondary syphilis the Wassermann test was positive in 6, the present method in 9.

Of 18 cases of manifest tertiary syphilis the two methods agreed and were positive in 17.

Of 18 cases of latent tertiary syphilis the Wassermann test was positive in 11, the present method in 14.

Of 11 untabulated cases of tabes dorsalis only 3 were positive with the Wassermann test and all were positive with the present test.

Of 28 cases suspected of syphilis or of syphilitic origin, the Wassermann test was positive in 16, the present method in 21.

Concerning certain points in the manner of employing the various reagents in testing the cases above reported, I may state that about one-third of all the cases were examined with liquid preparations

<sup>5</sup>The cases recorded in this table were derived chiefly from the clinics of Dr. Victor C. Pedersen of the New York Hospital and House of Relief, Dr. Howard Fox of the Vanderbilt Clinic, and Dr. J. A. Fordyce (through Dr. Homer F. Swift) of Bellevue Hospital. Ten of the suspected cases belong to Dr. Jacob Heckmann of the German Hospital Dispensary, and will be reported by him from a clinical standpoint in a later publication. It is my pleasant duty to acknowledge my indebtedness to these gentlemen for their assistance in furnishing me with the clinical material.

of complement, antigen and amboceptor, while a second third were tested in a similar manner except that the complement and amboceptor were first dried and pulverized before they were dissolved in saline solution for use. The remaining third of the cases were examined by the reagent paper method. The results obtained by this last method were perfectly reliable and satisfactory. It is needless to say that every one of these tests had been carefully controlled by the use of liquid reagents and by the regular Wassermann test.

In comparing the results obtained by the original Wassermann method with those obtained from the simple method here described, it should be pointed out that cases which were negative to the Wassermann and weakly or often quite strongly positive to the present method were met with. The causes of the observed differences were found in the existence of a large amount of natural amboceptor for sheep's blood corpuscles in such specimens of human serum. No disagreement in the result between the two methods was found with strongly positive specimens (containing more than eight units of syphilis antibody in 0.1 c.c.) or with weak specimens devoid of the natural amboceptor.

I would finally draw attention to the value of the test as a guide to the treatment of cases of syphilis. It appears that with the progress of the cure the symptoms of the disease not only abate, but the blood reaction grows weaker until ultimately when the cure has been established the reaction can no longer be obtained. If experience supports this result, the duration of the antisyphilitic treatment and its efficacy may become subject to quantitative control. The advantages which would accrue from such a measure are obvious. I mean to pay strict attention to this important therapeutic point and shall hope to report on a much larger series of cases in the near future.