

MICROSCOPIC AND SEROLOGIC TESTS FOR SYPHILIS*

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Laboratory tests for syphilis fall into two fields, microscopic and serologic. With few exceptions microscopic tests are of value only in the primary stage of the disease, whereas serologic tests are of great diagnostic aid in the late primary, the secondary and tertiary stages.

Microscopic tests may be either of stained or living preparations. By far the most satisfactory is the living or "hanging drop" preparation. Indeed, it is only by this method that a diagnosis is possible the very first day of the disease. The discovery by Siedentopf and Zsigmondy of the principle of the ultramicroscope led to the discovery, in 1905, by Schaudinn and Hoffmann, of the *treponema pallidum*, the causative organism of syphilis.

The finding of the *treponema pallidum* is an absolute diagnosis of syphilis. The search for this organism, whenever this is practicable, should never be omitted. The ease with which the so-called "darkfield" examination may be made lends itself to office procedure. All suspicious lesions should be examined, and if the *treponema* be not found, be reexamined. Very frequently the patient presents himself with a lesion that he has had treated

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with various medicaments for from several days to several weeks. The treponema is easily susceptible to antiseptics and germicides, consequently a treated lesion may, on first examination, yield no treponema. Subsequent examinations, however, favor the finding of the organism, particularly if a warm normal saline dressing be applied several hours daily for a day or two before examination. Of thirty-eight patients examined in our laboratory, who had been under very intensive local treatment, and who were negative for *T. pallidum* at the first examination, five on subsequent examinations yielded the specific organism.

In our experience about five per cent of the patients examined by the darkfield method have had no treatment whatsoever before examination. Alas, in many cases the physician himself instituted treatment, and then became suspicious of lues when the lesion failed to heal. The ease with which the treponema can be demonstrated in untreated lesions should be a warning never to treat a lesion until at least two negative findings have been obtained.

The lesions in the serum, of which it is possible to find the organism of syphilis with reasonable ease, are: primary sores; enlarged glands adjoining primary sores; all papular lesions of the skin, including condylomata; and secondary lesions of the mouth and throat. The lesion under examination should be carefully freed from extraneous contamination by gently swabbing with normal warm saline or water, and then scraping lightly. The oozing serum is then transferred with a sterile loop

to a slide, on which had been placed a loop of distilled water. Dilutions from this mixture may be made if too much blood is present. We have found a wax pencil ring made about the suspension to be as effective as a vaseline ring. The treponema in such a hanging drop preparation has remained viable for at least eight hours. A number one coverslip has given uniformly good results.

Search for the treponema in the hanging drop preparation should be thorough. In our laboratory at least three such preparations are examined, if the first slide be negative. Whenever possible, the microscope used for the darkfield examination should be used for no other purpose. Identification of the treponema pallidum should be made only by an experienced worker. In the examination of preparations from mouth lesions particular care must be used not to confuse the mouth spirochetes with the treponema pallidum.

At times, when the lesion itself yields no treponema, it may be advisable to puncture the enlarged gland. The secretion thus obtained will frequently show large numbers of the treponema.

DARKFIELD EXAMINATIONS 1924-1928

| | |
|--|------|
| Total males examined | 398 |
| Number positive for <i>T. pallidum</i> | 172 |
| Per cent positive for <i>T. pallidum</i> | 39.6 |
| Total females examined..... | 36 |
| Number positive for <i>T. pallidum</i> | 16 |
| Per cent positive for <i>T. pallidum</i> | 44.4 |

Table I

Table I indicates that in two out of every five persons examined the treponema could be demonstrated. Since no record was kept as to the char-

acter of the lesions, we are unable to state the number which actually were luetic. A fair percentage of persons who gave negative darkfield findings later gave serologic evidences of syphilis. It is well to emphasize the urgent necessity for repeated serologic tests, when darkfield examinations have proven negative.

Identification of the *treponema pallidum* in stained preparations should be made only by workers who have had considerable experience with the organism. Such preparations may be used as corroborative evidence of its presence. In our laboratory, if the smear from the suspected lesion be submitted and the organism found, a presumptive diagnosis of lues is made, leaving to the physician the task of making the final diagnosis. This he can make upon the appearance of the sore and, if necessary, upon the blood findings.

Where it may be impractical to send the patient to the laboratory, the serous exudate from the suspected lesion, collected and sealed in a capillary tube, may be sent. In one such preparation we found the *treponema pallidum* viable after eighteen hours transit through the mails.

SEROLOGIC TESTS

Since the finding of the specific organism is limited usually to the primary stage of syphilis, the importance of having a reliable laboratory test for all stages is at once apparent. Bordet and Gengou, in 1906, completed their studies on the phenomenon of complement fixation, and the adaptation of this test to syphilis was made soon thereafter by Wassermann, Neisser and Brucke. The Wasser-

mann test has proven to be one of the greatest factors in the recognition and control of syphilis. It is not only a reliable diagnostic test, but it also, when used quantitatively, indicates the therapeutic progress of the patient.

The Wassermann test for syphilis differs from other complement fixation tests in that the antigen employed is nonspecific. Wassermann originally employed as an antigen an aqueous extract of syphilitic liver which contained large numbers of spirochetes and their end-products. This antigen, however, was found to be rather unsatisfactory, and led to the use of extracts of other tissues, both syphilitic and normal. Alcoholic extracts of normal heart muscle have for some time been used almost universally as antigens, not only in the Wassermann test but also in the various precipitation tests for syphilis.

The complexity of the Wassermann test and the difficulties inherent in complement-fixation tests led many workers to modify the test to a greater or lesser extent. For a time there were as many modifications of the original Wassermann technic as there were workers employing the test. In 1922 Kolmer and his coworkers made a thoroughly comprehensive study of the Wassermann reaction and devised a modification of the test which undoubtedly is superior to all complement fixation tests for syphilis.

The complexity of the Wassermann test led workers also to develop new tests for syphilis. In 1907 Michaelis used an aqueous extract of syphilitic liver as an antigen, and syphilitic serum as the

antibody, and obtained a precipitate. In 1908 Jacobstahl, employing a diluted alcoholic liver extract, observed by means of the ultramicroscope the formation of floccules when it was mixed with syphilitic serum. Since then many workers devised precipitation tests, among which those of Meinicke, Sachs-Georgi, Dryer and Ward in Europe, and of Kahn in the United States stand out most prominently. The Kahn precipitation test, because of its high specificity and simplicity, has replaced, since January, 1929, the Wassermann test in the Oregon State Hygienic Laboratory as the routine serologic test for syphilis.

The Wassermann test requires five reagents: (1) the patient's serum; (2) the nonspecific antigen (alcoholic heart muscle extract); (3) complement (fresh guinea-pig serum); (4) hemolysin (immune rabbit serum); and (5) red blood cells (sheep or human). The Kahn test requires only two reagents: (1) the antigen (alcoholic heart-muscle extract); and (2) the patient's serum. The Kolmer test requires over-night incubation in the ice-box; the Kahn test may be completed within an hour, at the most, after the drawing of the patient's blood, or within a few minutes after the inactivation of the serum.

Medical literature has had many contributions on the Kahn precipitation tests. The writer reported comparative studies made on the Kahn and Wassermann tests in 1923¹ and 1924,² in both of which he concluded the Kahn to be the superior. A tremendously large number of comparisons between the Kahn and the Wassermann tests in many lab-

oratories has shown very close agreement between the two, and an increasing number of workers have reported the superiority of the Kahn test not only over complement fixation tests, but also over other precipitation tests. The most outstanding comparison between the Kahn and all other tests was made in 1928, when the League of Nations Health Committee arranged a competitive conference at the State Serum Institute in Copenhagen.

"The Conference considered four requirements as basic for a desirable test for syphilis.

"1. Practicability. A test to be practical must be so constructed as to be performed with comparative ease. If the test is of such character as to require elaborate and complex technical details or apparatus, it obviously would not stand high in practicability.

"2. Sensitiveness. A test should be capable of detecting as many cases of syphilis as possible.

"3. Specificity. It should not "pick up" any false positive reactions, or only a very minimum number of them.

"4. Clear-cut reactions. It should give as small a number as possible of doubtful or plus-minus reactions."³

The conference concluded that "the best of tests depending on precipitation may be regarded as equal in value to the best of tests depending on complement fixation." The Kahn test, besides being one of the five tests which were free from false positive reactions, "picked up" 305 positives against 208 positives given by the best of the Wassermann

methods, of which seven were used at this conference. With one exception the Kahn method yielded more positives than any other precipitation test, eight of which were used at the Conference. The Muller test, which obtained 317 positive reactions, lacks in practicability because the antigen can be obtained only from one pharmaceutical house. A full discussion of the various methods employed as well as the results obtained may be found in the Report of Second Laboratory Conference on Serodiagnosis of Syphilis, Geneva, League of Nations Health Organization, 1928.

From October, 1923, to January, 1929, the Kahn test was used in our laboratory in conjunction with the Wassermann test. Many bloods that were submitted had just enough serum for one test; consequently a larger number of Wassermann than Kahn tests were made. In spite of that, the number of positive sera picked up by the Kahn test was considerably larger than that picked up by the Wassermann. This is shown in table II.

RESULTS OBTAINED WITH KAHN AND WASSERMANN TESTS
1924-1928

Total bloods on which Wassermann test was made, 21,310.
Total bloods on which both Kahn and Wassermann tests were made, 20,741.

| | <i>Wassermann Test</i> | <i>Kahn Test</i> |
|----------------|----------------------------|----------------------|
| Positive | 2,900 | 3,408 |
| Doubtful | 1,533 | 1,605 |
| Negative | 16,877 | 15,728 |

Table II

Since January, 1929, the Kahn test alone has been used in our laboratory. For the first six

months a total of 3382 sera was tested. Table III shows the comparative percentages of the results obtained with the Kahn test for this six month period as against those obtained with the Wassermann test for five years.

RESULTS IN PERCENTAGES, KAHN AND WASSERMANN TESTS

| | <i>Wassermann test (21,310 sera)</i> | | | | | <i>Kahn test (3382 sera) 1929 (6 mos.)</i> |
|----------|--|------|------|------|-----------|--|
| | 1924 | 1925 | 1926 | 1927 | 1928 Mean | |
| Positive | 15.5 | 12.5 | 12.9 | 14.4 | 13.1 | 20.2 |
| Doubtful | 7.1 | 6.0 | 5.5 | 8.9 | 7.7 | 5.2 |
| Negative | 77.4 | 81.5 | 81.6 | 76.7 | 79.2 | 74.6 |

Table III

A study of table III indicates that the Kahn test has yielded more positive results than have been obtained in any year previously with the Wassermann test.

The Kahn test is being made on all spinal fluids received for serologic examination. While the technic is not so simple as for the blood sera (a preliminary precipitation of the globulin is necessary), it also yields excellent results. In table IV the results obtained with the Wassermann test in the five-year period on spinal fluids are compared with those obtained with the Kahn test for the six-month period.

RESULTS WITH SPINAL FLUIDS

| | <i>Wassermann test 1924-1929</i> | | <i>Kahn test 1929 (6 mos.)</i> | |
|----------|--------------------------------------|-----------------|------------------------------------|-----------------|
| | <i>Number</i> | <i>Per cent</i> | <i>Number</i> | <i>Per cent</i> |
| Positive | 79 | 11.6 | 25 | 22.5 |
| Doubtful | 56 | 8.2 | 16 | 14.4 |
| Negative | 548 | 80.2 | 70 | 63.1 |

Table IV

Here, again, the superiority of the Kahn test is plainly evident. In a special competition held at London in July, 1928, with the Harrison-Wassermann method on tests with spinal fluids, Kahn was able to demonstrate the reliability of his test. Of 508 spinal fluids examined the Wassermann test gave a total of 177 ++ and 63 + reactions, while the Kahn gave a total of 233 ++ and 18 + reactions. Neither method gave false positive reactions.

The Kahn test performed in our laboratory is carried out according to the technic described by Kahn in his book, "The Kahn Test, A Practical Guide."⁴ Two antigens, a standard and a special (very sensitive) are used. Every specimen, therefore, is checked at least once before final results are recorded. Since the antigen is the key-stone of both the Wassermann and precipitation methods, every lot of antigen is very carefully standardized. Uniformity of procedure is absolutely essential. We have checked a few of the Kahn results of one laboratory, where there was a rather marked disagreement with the Wassermann results, and found that the trouble was in the improper standardization of the Kahn antigen. Our own standard antigen always gave comparable, and at times stronger reactions than the Wassermann test.

The Kahn test should be carried out by serologists, hence it should not be the plaything of the office girl. It is true that it is a very simple laboratory procedure, but because of its simplicity it requires an alert mind and skilled hands to manipulate it.

We had some misgivings as to the reaction of the Oregon physicians to the substitution of the Kahn for the Wassermann test. We have not had a single complaint, and from the increasing number of specimens received one must conclude that they have taken the Kahn test strongly to their hearts. We run the Kahn test daily, hence reports are received by physicians from one to five days earlier than when the Wassermann test was run. We have done away with all laboratory animals previously employed in the Wassermann test, and have, therefore, reduced the cost of the serologic tests made by our laboratory.

SUMMARY

1. All suspicious lesions should be examined by the darkfield. Out of 434 persons examined, 188 or 43 per cent yielded the *treponema pallidum*.
2. Never treat a lesion until after two negative dark-field examinations have been obtained. Such negative microscopic findings must always be checked by serologic tests.
3. Since January, 1929, the Kahn test alone has been the routine test for syphilis in the Oregon State Hygienic laboratory. Based on our findings since 1924, in over 20,000 specimens tested (including sera and spinal fluids), the Kahn test has consistently "picked up" more positive reactions than the Wassermann.
4. The Kahn test should be performed only in laboratories equipped with proper apparatus and well-trained personnel.

State Hygienic Laboratory.

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