IX

THE LAUGHLEN RAPID TEST FOR SYPHILIS

A NEW TECHNIQUE EMPLOYING GARROW'S AGGLUTINOMETER

By A. H. WALTERS,

Pathological Laboratory, Albert Dock Hospital, London.

LAUGHLEN * has presented a reliable test for syphilis which can be performed in a few minutes, needs little equipment, no tubes and is inexpensive. It is especially adapted for routine use in small hospitals and clinics and for emergency use. The Laughlen test has as its base a precipitation test antigen, as used in the Kahn test, to which has been added balsam and Scarlet R. dye. The water insoluble dye colours the suspended particles of the antigen, but does not colour to any extent the liquid in which they are suspended, thereby increasing the visibility of a positive reaction.

The Laughlen reagent (antigen), which is supplied in the inactive state to insure the maximum stability of the antigen, is stable for a minimum of six weeks. It is stored normally at room temperature or, in hot weather, in a cool place. For activation 1 c.c. of the reagent is measured into a tube and to this is added 10 per cent. saline in the quantity stated on the label, usually 0.15 c.c. The fluid is mixed by shaking and is set aside at room temperature for twenty-four to forty-eight hours.

TEST MATERIALS

- (1) Activated Laughlen reagent (antigen).
- (2) Capillary pipette made to deliver approximately 0.013 c.c., the two divisions indicating this amount being marked on the stem, the lower mark at least $\frac{1}{2}$ inch from the tip.
- (3) 0.2 c.c. pipette of Kahn test pattern.

* Laughlen, G. S. : *Canadian Med. Assoc. J.*, 1935, **33**, 179.

THE LAUGHLEN RAPID TEST FOR SYPHILIS

- (4) Known positive and known negative sera.
- (5) A small dish containing fresh physiological saline for washing out the pipette.
- (6) Garrow's agglutinator.* † ‡

TECHNIQUE

- Note :* (a) Not more than 28 tests, including positive and negative controls can be made at one time.
- (b) Total time for 28 tests about fifteen to twenty minutes.
- (c) Sera for test should be numbered 1 to 26; N, and P.
- (d) It is essential that the technique once begun be carried out as quickly as possible to prevent evaporation of the drops.
- (1) 0.2 c.c. of Laughlen reagent is drawn up into 0.2 c.c. pipette Kahn test pattern.

SKETCH I

(2) The pipette is held vertically and the bottom gently lowered on to the first division of the glass table of the agglutinator, and a 0.01 c.c. drop released. This procedure is repeated on each division of the glass table to the number of tests—not exceeding 28—to be carried out at one time. The drops should be placed as shown in Sketch 1.

(3) With a teat capillary pipette 0.013 c.c. approx. of patient's inactivated serum (No. 1 specimen) is delivered

* "Manson's Tropical Diseases," 10th ed. Cassell, 1935.

† Garrow, R. P. : *J. Roy. Army Med. Corps*, 1917, **28**, 566.

‡ Supplied by Messrs. Baird & Tatlock Ltd.

on to the first division of the glass table of the agglutino-meter a little above the drop of Laughlen reagent, as shown in Sketch I. The 2 drops should remain discrete.

(4) The teat pipette is now washed out rapidly twice with saline, emptied, and the outside wiped dry.

(5) No. 2 sample of serum for test is now picked up and a small quantity of serum first sucked up into the pipette and expelled to wash out any adherent saline, then procedure as in step 3 repeated, the 0.013 c.c. drop of serum being deposited on No. 2 division of the glass in the manner shown in Sketch B.

(6) Step 4 is now repeated and each serum treated in the manner outlined. The glass table now has first a row of pink drops of reagent and behind that a row of serum drops.

SKETCH II

(7) The glass table is now set rotating by releasing a catch in the clockwork attachment. If, after twenty seconds, 1 or 2 of the drops of serum and reagent have not mixed, they should be assisted, as the table rotates uppermost, by very rapidly placing a clean needle in the serum drop and dragging it down to the reagent drop. This is quite easily done without stopping rotation after a little practice.

(8) After five minutes' rotation, the machine should be stopped and the glass table held horizontally with a hand at each end, slid out and inspected. Divisions showing a red precipitate show a positive reaction. The numbers (specimen) of the positives should be rapidly noted. (When reading the glass table in daylight the worker should hold the table away from the bench

THE LAUGHLEN RAPID TEST FOR SYPHILIS

because the red precipitate shows up better against the darker floor. When reading at night light directed through the glass table from underneath makes reading very easy (Sketch II.).)

(9) The glass table is at once replaced and the machine allowed to rotate for a further three and a half minutes and the reactions read off again with the aid of a magnifying glass. (Ophthalmic binoculars which can be attached to the head, leaving the hands free, are very useful for the purpose.) Attention after the final rotation should be directed to the negative control, and if this shows the slightest sign of precipitate then the "five minutes" reactions should be accepted with reserve. This occurs very rarely and usually when a batch of reagent is used after the maximum period of fourteen days from activation with saline.

REMARKS

(1) The Laughlen test technique as formulated by the originator employs the use of slides as in ordinary blood grouping. One drop of reagent plus one drop of serum slightly in excess of the reagent are mixed by tilting the slide repeatedly, and then observed at intervals up to ten minutes for the presence of precipitate.

The modified technique described here gives:—

- (a) A known quantity of reagent, 0.01 c.c.
- (b) A known quantity of serum, 0.013 c.c.
- (c) A known mixing rate: the agglutinator revolves fifteen times a minute.
- (d) Set reading times. Positive at five minutes, weak or doubtful at nine minutes.
- (e) Increased facility for doing numbers of tests.

(2) Laughlen states that "Agglutination is approximately the same whether it be a strongly positive or a weakly positive reaction." By the modified technique this is not found to be the case, and the degree of positivity is judged, after some experience, by the amount and size of the granules. As in the Kahn test the coarser the granules, the more positive the specimen. It is advisable that control Kahn tests be carried out on the first 300 sera tested to enable the observer to classify Laughlen results accurately in terms of the Kahn test for degree of positivity. The positives after five minutes can be interpreted in terms of four plus or three plus Kahn,

and the weak or doubtful positives after nine minutes can be interpreted in terms of two plus or one plus Kahn.

(3) The modified technique must be a strictly standardised procedure. Workers are advised to carry out not more than 6 tests at a time until they have become really expert, after which they can gradually increase the number done at a sitting.

(4) It is strongly advised that the technique be carried out in a cool, clean room, if possible, and not in a general laboratory.

(5) After the agglutinator has been set in motion and all the drops are mixing well (usually about twenty seconds) the agglutinator box may be closed until the first five minutes rotation is up. This tends to reduce evaporation and to exclude outside contamination.

(6) In weak or doubtful positives the red precipitate, composed of very fine granules, appears along the edges of the mixed drop on the glass table.

(7) It has been found that various batches of the activated reagent vary slightly in the speed of producing positive results. This slight variation may be ignored since the first reading time, five minutes, is ample time for the slightly slower batches to produce positive results.

RESULTS

Four hundred sera have been tested. Ten c.c. of blood was withdrawn from each of 400 consecutive patients as they attended the V.D. Clinic at Albert Dock Hospital, directed by Dr. H. M. Hanschell. Each serum was tested by Kahn and Laughlen test (modified technique). Of the 400 cases, 57 were diagnosed clinically by Dr. Hanschell as syphilis, and 343 as not syphilis.

Table I. shows results obtained on the 57 cases diagnosed as syphilis. The two primary syphilis cases which gave Kahn negative and Laughlen negative results were early sero negative cases diagnosed clinically and by the presence of spirochaetes in the primary lesions. Of the remainder of the primary syphilis cases, 1 gave a Kahn negative and a doubtful Laughlen test, another a doubtful Kahn and a Laughlen negative, and another a doubtful Kahn and doubtful Laughlen test. These 3 cases when tested three weeks later all gave four plus Kahn and Laughlen test results. Eighteen cases of primary syphilis gave positive Kahn and Laughlen test results.

THE LAUGHLEN RAPID TEST FOR SYPHILIS

TABLE I

Disease	K. & L. agree	K. Neg. L. W. or D.	K. W. or D. L. Neg.	Total
Early syphilis (less than 1 year)	34	1	1	36
Late syphilis (more than 1 year)	7	0	*1	8
Treated syphilis . . .	12	0	*1	13
Total . . .	53	1	3	57

K. = Kahn.
W. = Weak.

L. = Laughlen.
D. = Doubtful.

All 5 cases of secondary syphilis, and all 8 cases of syphilis detected within the first year of infection, gave positive Kahn and Laughlen test results.

One case of syphilis of more than one year's standing gave a weak positive Kahn and a negative Laughlen test result. Repeat tests on this case gave the same result persistently. The patient was a Lascar who could not speak English and may have had treatment previously.

Seven cases of syphilis of more than one year's standing gave positive Kahn and Laughlen test results.

Out of a total of 13 cases of syphilis known to have received treatment elsewhere 8 cases gave positive Kahn and Laughlen test results, 4 gave weak or doubtful Kahn and Laughlen test results, and 1 gave doubtful Kahn and negative Laughlen test result. The latter case had received two full courses of arsenobenzen treatment, and the 4 cases giving weak positive reactions had received the equivalent of at least one course of arsenobenzen treatment and were recovering.

From an analysis of the total of 57 cases in this group of syphilis cases, it appears that the Kahn is in complete agreement with the Laughlen test in all the positive cases of syphilis, but that the Kahn is very slightly more sensitive in registering weak or doubtful reactions in old cases than is the Laughlen test (*vide* cases marked with asterisk).

Table II. shows results obtained on the 343 not syphilis cases; 335 sera gave completely negative Kahn and Laughlen test results; 6 sera gave a negative Kahn and a doubtful Laughlen test result. Of these 6 tests fresh

TABLE II

No. of Not Syphilis Cases	K. Negative L. Negative	K. Negative. L. Weak or Doubtful	K. Weak (+ + - -) or Doubtful (+ - - -) L. Negative.	K. + + + - or + + + + L. + + + - or + + + +
343	335	6	2	0

serum was obtained from 4 of the cases, and all 4 repeat tests gave negative Kahn and negative Laughlen test results. Sera for repeat test from the remaining 2 cases could not be obtained for repeat test.

Two sera gave a doubtful Kahn and a negative Laughlen test. Opportunity to repeat tests on these 2 cases was not available. No serum gave a positive Kahn or a positive Laughlen test result in the series of not syphilis cases.

From an analysis of the totals of the 343 not syphilis cases in this group it would appear that the Laughlen test may give a doubtful reaction in 1.7 per cent. of not syphilis cases against 0.6 per cent. with the Kahn test carried out in this laboratory. Since repeat tests on 4 out of 6 sera which at first returned doubtful Laughlen test results subsequently proved negative, it is quite possible that some foreign substance entered the blood serum from the syringe during collection from the patient, or from the storage tube in which it was transported to the laboratory, which gave rise to a doubtful result.

CONCLUSION

From the results obtained on 400 sera from consecutive patients the Laughlen rapid test for syphilis (modified technique, would appear to be a very reliable test for syphilis cases that normally give a Kahn three plus or Kahn four plus result. Since it is usual to accept doubtful positive results in syphilis sero-diagnosis by any test with reserve, the Laughlen test (modified technique) may be considered a useful general rapid test for syphilis subject to later confirmation by the Kahn test in all sera giving a doubtful positive result.

My thanks are due to Dr. H. M. Hanschell, Director of the V.D. Clinic, for facilities for carrying out this research.