THE LAUGHLEN TEST

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REFERENCES

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"Primer on Arthritis" (1942) J. Amer. med. Ass., 119, 1089.

THE PRACTICAL AdvANTAGES OF THE LAUGHLEN TEST FOR THE DIAGNOSIS OF SYphilis.*

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The Laughlen test for syphilis was developed as the result of a search for a method better adapted to the requirements of a busy hospital laboratory than were those methods which existed in 1935. The particular need was for one that could be completed in a short time to enable a laboratory to rule out syphilis in a blood donor. None of the methods in use at that time could qualify as acceptable for that purpose. The Wassermann reaction was far too complex for any but the largest laboratories, and tests such as the Kahn presented difficulties of interpretation that required the services of expert serologists. It was true that the city and provincial health laboratories provided a very reliable and efficient service, but this was not entirely adequate, because reports could seldom be secured inside of two or three days and often longer. This service did not supply all the needs of city hospitals, and those outside large centres such as Toronto were having even more difficulty in getting quick reports.

Historical note

The Laughlen test was introduced to the medical profession in August 1935 (Laughlen1). In the original paper the preparation of the reagents and the technique were described in detail, and a series consisting of approximately 1,000 comparative tests was reported. In a second paper (Laughlen 2) some changes in the preparation of the reagents and in the technique were described and a report was made on a series of over 5,000 comparative tests. A series of 63 tests, done on citrated plasma, was also reported; this report showed that if the citrate concentration were kept at a minimum, plasma could be employed with the

* An address to the Annual Meeting of the Ontario Medical Association, 23rd May 1945.
Laughlen method. Since the publication of the original paper, upwards of sixteen articles have appeared in the medical literature (see the names marked with an asterisk in the References on p. 20). They have been for the most part records of surveys of Laughlen and other test methods for the detection of syphilis in blood serum. They represent upwards of 30,000 tests. There have also been State and National Public Health Surveys in the United States of America in

### TABLE 1—RESULTS IN 22 CASES (OUT OF 1,293) IN WHICH TESTS AGREED INCOMPLETELY

<table>
<thead>
<tr>
<th>Specimen number</th>
<th>Wassermann</th>
<th>Kahn</th>
<th>Laughlen</th>
</tr>
</thead>
<tbody>
<tr>
<td>119</td>
<td>+</td>
<td>+</td>
<td>D</td>
</tr>
<tr>
<td>211</td>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>344</td>
<td></td>
<td>-</td>
<td>D</td>
</tr>
<tr>
<td>358</td>
<td></td>
<td>-</td>
<td>D</td>
</tr>
<tr>
<td>409</td>
<td></td>
<td>-</td>
<td>D</td>
</tr>
<tr>
<td>548</td>
<td>D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>554</td>
<td></td>
<td>-</td>
<td>D</td>
</tr>
<tr>
<td>590</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>802</td>
<td>D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>827</td>
<td></td>
<td>-</td>
<td>+ (treated)</td>
</tr>
<tr>
<td>859</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>898</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,041</td>
<td>D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>115</td>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>123</td>
<td>D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>124</td>
<td>-</td>
<td></td>
<td>+ (treated)</td>
</tr>
<tr>
<td>346</td>
<td>*</td>
<td>-</td>
<td>D</td>
</tr>
<tr>
<td>364</td>
<td>D</td>
<td></td>
<td></td>
</tr>
<tr>
<td>410</td>
<td></td>
<td>-</td>
<td>D</td>
</tr>
<tr>
<td>444</td>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>530</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>533</td>
<td>D</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

D=doubtful.

which the Laughlen test has been subjected to comparison with other methods (Perry; Parran and others).

The National Public Health Survey (Parran and others) which was held in Washington in October 1941 was along much broader lines than those of the State surveys (Perry). It was primarily a comparison of the accuracy and sensitivity of the different serological methods under favourable conditions. The testing was conducted by expert technicians. The survey yielded valuable information as to the effect of diseases other than syphilis upon the accuracy of the results by the different methods.

### Advantages of the test

The published papers and records of surveys, as quoted above, afford abundant evidence that the Laughlen test is a sensitive and accurate method for the detection of syphilis in blood serum. It may be used for emergency testing when one or more samples are examined on slides, or it may be used to conduct series of tests. When so used, larger ruled-glass plates are more convenient. The details of the
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technique have been described previously (Laughlen 1, 2, 3). It has been used more extensively as an emergency or exclusion test, but its value as a routine laboratory procedure appears to have been partially overlooked. It saves time and provides reports on the day on which the samples are taken, or within a few minutes in cases of emergency. When it is used as a routine measure, tests are performed in one series, or better still in two series which are made simultaneously. In one of these series a highly sensitive antigen designed to detect minor degrees of sensitivity in the samples is employed. (Two series of tests can be made almost as easily as one.) By this means the possibility of failure to detect syphilis can be almost eliminated, and re-checking by other methods becomes unnecessary. If a hospital laboratory adopts this plan and accepts the negative reports as final, further testing or re-checking is needed only for doubtful or positive samples and they constitute only a small percentage of the total. The saving of laboratory services is considerable. The positive samples should be dealt with as follows: each serum sample is diluted with saline in the proportions of 1 in 2, 1 in 4, 1 in 8 and so on, and retested by the Laughlen method.

The quantitative method of testing, which includes a simple way of diluting the serums, has been described (Laughlen4). This can be done in a few minutes' time and gives a means of measuring the degree of positivity of the samples. The figures (4 times the dilution) have the same value as have Kahn units. Blood samples which are either doubtful or positive are submitted for testing by other methods.

It is established practice that the diagnosis of syphilis should not be made on the basis of a single positive test by any method; at least two methods must be employed; the use of more than two or three does little to improve the accuracy of serological testing.

The Laughlen test is adapted to use in out-of-the-way places which are without the services of a central laboratory. By the use of Laughlen antigen, which can be kept indefinitely, test reagents can be made as needed without difficult technical procedures. The test has been used with success on board battleships, and the advantages of its use on board any large ocean-going vessel are evident.

Tests on ward samples

The above technique has been employed in the Toronto East General Hospital since June 1944, and upwards of 1,293 ward patients and about 400 special

<table>
<thead>
<tr>
<th>PUPIL TECHNICIAN</th>
<th>NUMBER OF BLOOD SAMPLES</th>
<th>PARTIAL DISAGREEMENT</th>
<th>NUMBER OF ERRORS</th>
<th>PERCENTAGE LIMITS OF ERROR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miss A.</td>
<td>1,000</td>
<td>7</td>
<td>3</td>
<td>0.3 - 0.1</td>
</tr>
<tr>
<td>Miss W.</td>
<td>1,000</td>
<td>19</td>
<td>4</td>
<td>0.4 - 2.3</td>
</tr>
<tr>
<td>Miss C.</td>
<td>383</td>
<td>3</td>
<td>1</td>
<td>0.3 - 1.2</td>
</tr>
<tr>
<td>Miss Mcl.</td>
<td>753</td>
<td>6</td>
<td>6</td>
<td>0.8 - 1.6</td>
</tr>
<tr>
<td>Miss M.</td>
<td>1,000</td>
<td>10</td>
<td>2</td>
<td>0.2 - 1.2</td>
</tr>
<tr>
<td>Miss H.</td>
<td>279</td>
<td>4</td>
<td>1</td>
<td>0.36 - 1.8</td>
</tr>
<tr>
<td>Miss T.</td>
<td>200</td>
<td>3</td>
<td>1</td>
<td>0.5 - 2</td>
</tr>
<tr>
<td>Miss R.</td>
<td>800</td>
<td>5</td>
<td>1</td>
<td>0.2 - 1</td>
</tr>
<tr>
<td>Miss Mcl.</td>
<td>1,293</td>
<td>12</td>
<td>4</td>
<td>0.3 - 1.3</td>
</tr>
</tbody>
</table>

* One test doubtful and the others either positive or negative.
† The smaller figures represent approximately the actual error.

treatment patients have had their blood examined. The tests were performed by a graduate technician, Miss Mcl. The 1,293 ward cases provided 22 samples in which there was not complete agreement between Wassermann, Kahn and
Laughlen tests; complete agreement was secured in over 98.3 per cent. The detailed results on the 22 samples in which there was incomplete agreement are shown in Table 1.

**Percentage of error in Laughlen and Kahn tests**

An analysis, on the basis of one test being in error which disagrees with the other two, shows an error of less than 1 per cent for either the Laughlen or the Kahn tests. (The Wassermann is not strictly comparable because too few tests were performed.) The Laughlen test showed a higher number of positives than did the Kahn. The degree of accuracy in this survey for the Laughlen test exceeds 99 per cent. An acceptable degree of accuracy for diagnostic tests would be a minimum of 99 per cent (when treated cases are not included). Exclusion tests would be acceptable if they did not fail to detect syphilis in more than 1 per cent of positive samples. Technicians of average experience and ability can perform routine Laughlen tests without exceeding an error of 1 per cent. This was demonstrated by surveys conducted in the East General and St. Joseph's Hospitals, Toronto. The results obtained by nine technicians in these surveys are shown in Table 2.

**Testing of small samples**

These surveys have shown that for routine as well as for emergency testing the Laughlen test is dependable, and that it is not too complex for use in a hospital laboratory which employs technicians. Another advantage over other methods is that it can be performed on as little as one or two drops of serum. This makes possible the testing of infants or others from whom venous blood cannot be obtained. Sufficient blood for the Laughlen test can be obtained from the heel or the great toe by the ordinary skin puncture. The puncture must be sufficient to provide 6 or 7 drops of blood, which are collected into an ordinary Wassermann tube (about 10 × 0.5 centimetres) the inner surfaces of which have been wetted with fresh 5 per cent sodium citrate solution (in physiological saline). Any excess of the citrate solution is removed by inverting the tube and allowing it to drain. (Excess of citrate will render the specimen unfit for testing.) This method of collecting blood for Laughlen tests should never be employed when venous blood is available.

**Sensitivity of the Laughlen test**

A blood test to be acceptable must be sensitive as well as accurate. It has to show an acceptable degree of sensitivity; if it is too sensitive, false positives will result. What is desired of a method is that it possess the full degree of sensitivity but not an excess that leads to errors. In order to determine whether a method is or is not satisfactory, the only means available is to compare its sensitivity with that of others which are recognized as dependable. This is done by conducting series of tests by the two or three methods under comparison on samples of blood from patients with syphilis. The Laughlen test has on many occasions been subjected to such comparisons and has usually shown a slightly higher degree of sensitivity than have the commonly accepted methods. In the Toronto East
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General Hospital, 362 samples from the Out-Patient Department (mostly from the Special Treatment Clinic) have afforded one such survey. The Laughlen test on these samples showed more positives and fewer negatives than did the Kahn; Fig. 1 shows in a graph the percentages obtained.

Another survey was made by Beck on 100 samples from cases under treatment. The Kahn, Wassermann and Laughlen tests were compared, and the results showed more positive, fewer doubtful and fewer negative reactions for the Laughlen than for either of the other two. The percentages are shown graphically in Fig. 2.

Hamilton-Paterson, Cole and Usher performed 12,865 slide tests (modified Laughlen) and compared them with Wassermann and Kahn tests on the same specimens. On 340 samples from probable syphilitics, they estimated the sensitivity of the Wassermann as 94·4 per cent, Kahn 91·74 per cent and the slide test 98·68 per cent. In their concluding remarks they state: ‘This sensitivity, combined with great rapidity and simplicity, renders the test most suitable for the preliminary sorting out of large numbers of sera, thus saving much dull routine work.’ Readers of their article would no doubt be impressed with the excellence of the work but might also gain the impression that many difficulties arise in the performance of the Laughlen test. This is not so if one starts with a satisfactory antigen or with the stable stock reagent. Laboratories have been advised not to prepare Laughlen antigen in the small quantities which they would consume, as the difficulties of standardization are considerable. They would save themselves much technical work if they would purchase the antigen ready-prepared from a central distributing laboratory. They would thus assure themselves of uniform reagents (tested and approved by the originator of the method). These authors used horse serum to lessen oversensitivity. The use of properly selected antigen would not necessitate this procedure. It is much safer to start with a correct (not oversensitive) antigen.

![FIG. 2—SENSITIVITY OF LAUGHLEN TESTS.](http://sti.bmj.com/)

Tests made of 100 sera from known syphilitic patients from the Department of Dermatology, University of Virginia. The graphs on the left represent tests made at the Stuart Circle Hospital, those on the right tests made at the University.

In the Washington survey of 1941 (Parran and others) the Laughlen test was compared with 27 other methods on 1,000 samples, of which 376 were from cases of syphilis. In this survey the Laughlen test, in common with all other methods, gave a high percentage of positive results on samples from leprosy and malarial cases. It showed, as did most of the test methods, a tendency to false positive results in samples from patients with cancer, with fever and convalescent from diseases with fever. With samples from tuberculosis cases it was 100 per cent
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accurate; with samples from normal donors it was 99·6 per cent accurate. With syphilitic blood samples it gave as high an incidence of positive tests as did methods with an equal or comparable degree of specificity.

Conclusions
The Laughlen test, which is simpler to perform, is not subject to errors in any greater degree than are other methods. It gives the same information with less work, less expense and in a shorter time. Some of its advantages are summarized below.

(1) It is adapted for the purpose of testing all routine blood samples in hospitals.
(2) Negative findings can be accepted as approximately 100 per cent accurate, thus eliminating the necessity for further testing.
(3) By its routine use reports are available within a few hours of the time at which samples are obtained.
(4) Tests of an urgent character can be made in 10 minutes after the blood serum is ready for testing.
(5) The test can be carried out on finger blood when venous blood is difficult to obtain, as, for example, in babies and in some adults.
(6) It is suitable for use on hospital ships, battleships or other large ocean-going vessels.
(7) It can be used in outposts which are without the services of central laboratories.
(8) Its sensitivity and accuracy are equal to those of the more complex methods.
(9) A quantitative method of analysis is provided by its use.

REFERENCES

* Kluge, Marie E. (1938) Personal communication, report on 1,065 Laughlen Tests compared with Kahn and Kolmer-Wassermann.
* For use of asterisk, see page 16.

Experimental treatment of syphilis with penicillin

According to Bessemans and Deron (writing in Bruxelles-médical for 23rd September 1945) infection of rabbits with syphilis appears to be effectively cured by repeated injections of penicillin. Rabbits experimentally infected with Spirochaeta pallida were treated with 320,000 units (12,000-18,000 units given intramuscularly every 3 hours) for a period of 8 days. It was found that S. pallida disappeared from the lesions in 1-3 days and that healing of the lesions—testicular syphilioma or keratitis, of 2-12 months' duration—was effected in 5-30 days. Larger amounts were given to some of the affected animals but apparently without any advantage in the rate of healing. At the end of 3-4 months a popliteal gland was transferred to a normal rabbit with subsequent negative results, indicating that the infection had been sterilized effectively. Similar experiments showed that latent syphilitic infection of 3-4 months duration in 8 mice responded to penicillin in a dosage of 12,000-18,000 units given over a period of 8 days.