THE RELIABILITY OF THE WASSERMANN TEST AS PERFORMED BY DIFFERENT PATHOLOGISTS.

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The object of the work here recorded was to discover in how far the results of Wassermann tests, performed by pathologists working under the various Venereal Diseases Treatment Schemes of the Local Government Board in Ireland, agreed with one another. The authorities of five pathological laboratories, in which 88 per cent. of all the Wassermann tests done under the schemes last year were performed, kindly consented to co-operate in this experiment. These pathologists, to whom my best thanks are due for their help and interest, are subsequently described as *A*, *B*, *C*, *D*, and *E*, in order to preserve their anonymity.

Samples of blood were obtained from thirty patients, attending Venereal Diseases Treatment Centres. To the medical officers of these centres—at Sir Patrick Dun's Hospital and Dr Steeven's Hospital, Dublin, the Royal Victoria Hospital and the Mater Infirmorum Hospital, Belfast—my thanks are due for their kindness in obtaining for me the necessary blood and also for the clinical details of the cases.

In Table I the cases are arranged in six groups, in accordance with their clinical condition.

Table I.

Group	Clinical diagnosis	Numbers
1	Primary syphilis (undergoing treatment)	2, 4, 7, 8, 12, 20, 24, 26, 27
2	Secondary syphilis (untreated)	1, 22
3	Secondary syphilis (undergoing treatment)	3, 6, 9, 10, 13, 14, 15, 16, 17, 18, 19, 21, 25, 28, 29, 30
4	Syphilis with involvement of central nervous system (undergoing treatment)	5
5	Clinically doubtful (untreated)	23
6	Clinically not syphilis (untreated)	11

The patients were all males. All treated cases had received injections of Novarsenobillon intravenously and, in the majority of the cases, mercury cream intramuscularly.

The samples of blood were taken in my presence and handed over to me. I separated the serum and divided it into five parts, one of which was sent to each pathologist, under a number of my own. In no case, therefore, did

the pathologist know anything of the patient from whom the serum came. Four batches of sera were sent by post to each of the five pathologists. Batch 1 consisted of the sera of patients 1 to 7, batch 2 patients 8 to 14, batch 3 patients 15 to 23 and batch 4 patients 24 to 30. An interval of one or more weeks elapsed between the collection of each batch of bloods. The pathologists reported their results to me as soon as their examinations of each batch had been made.

Before giving the results the method of performing the Wassermann test employed by the collaborators will be indicated.

Pathologist A.

The method is that described by Harrison (method No. 1, Medical Research Committee's Report, No. 14, 1918).

The results are recorded thus:

++
+±
+
±
$\frac{\pm}{-}$

Pathologist B.

The method is that of Harrison, except that the antigen is one prepared by Messrs Burroughs, Wellcome and Co. It consists of human heart extract and cholesterol in one solution. The results are recorded in the same way as those of pathologist *A*.

Pathologist C.

Harrison's method is slightly modified by *C*. The antigen used is a bullock heart extract containing 0·4 per cent. of cholesterol. It is used in a dilution of about 1/30, the exact dilution being determined by titrations of its power of inhibiting the action of complement in the presence of and in the absence of a positive Wassermann serum. The tubes contain the following reagents:

	Tube 1	Tube 2	Tube 3 (control)
Patient's serum ...	0·1 c.c.	0·1 c.c.	0·2 c.c.
Antigen (1/30) ...	0·5	0·5	—
Saline	—	—	0·5
Complement (3 M.H.D.)	1·0	—	1·0
Complement (5 M.H.D.)	—	1·0	—
Fixation, 1 hour in water bath at 37° C.			
Sensitised cells ...	1·0	1·0	1·0

The results are recorded thus:

++
<++
+
±
$\frac{\pm}{-}$

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Pathologist D.

The method is that of McIntosh and Fildes (*Syphilis from the Modern Standpoint*, 1911). This is a "one tube" method. That is, there is only one tube containing patient's serum, complement and antigen. A second tube containing serum and complement acts as a "serum control." The antigen is an alcoholic extract of human heart, without cholesterol. (McIntosh and Fildes now employ an antigen which contains cholesterol.) The tubes contain:

	Tube 1	Tube 2 (control)
Saline	0·74 c.c.	0·8 c.c.
Antigen (undilute) ...	0·06	—
Patient's serum ...	0·1	0·1
Complement (2½ M.H.D.)	0·1	0·1
Fixation, 1 hour at 37° C.		
Sensitised cells ...	0·5	0·5

The necessary controls for antigen, complement, etc., are also set up. The results are recorded thus:

+4
+3
+2
+1
Negative

Pathologist E.

This is a two tube method, two antigens being used.

Antigen 1. Alcoholic extract of human heart. Diluted 1/25.

Antigen 2. The same with 0·4 per cent. cholesterol added. The tubes contain:

	Tube 1	Tube 2	Tube 3 (control)
Patient's serum ...	0·1 c.c.	0·1 c.c.	0·2 c.c.
Complement (2 M.H.D.)	0·5	0·5	0·5
Antigen 1 (1/25) ...	0·5	—	—
Antigen 2 (1/25) ...	—	0·5	—
Saline	—	—	0·5
Fixation, 1 hour at 37° C.			
Sensitised cells ...	0·5	0·5	0·5

The results are recorded in the same way as those of *D*.

Owing to the different notations in use by these five pathologists, it became necessary to reduce them to a common expression. This was accomplished

Table II.

Suggested symbol	Interpretation	*A* and *B*	*C*	*D*	*E*
N	Negative	−	−	−	−
NP	Some inhibition. Insufficient for diagnosis but useful in controlling treatment	±, ±	±, ±	+1, +2	+1
P	Definitely positive	+	+	+3	+2
PP	More strongly positive	+±, ++	<++, ++	+4	+3, +4

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by using letters as symbols. Table II shows the individual symbols used by each worker and those adopted in the new method.

The next table (III) gives the results of the tests all reduced to the new system. In the last column the majority result is stated.

Table III.

Serum	*A*	*B*	*C*	*D*	*E*	Majority result
1	*PP*	*NP*	*PP*	*PP*	*PP*	*PP*
2	*PP*	*N*	*PP*	*PP*	*PP*	*PP*
3	*PP*	*PP*	*PP*	*P*	*PP*	*PP*
4	*N*	*N*	*N*	*N*	*N*	*N*
5	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*
6	*PP*	*PP*	*P*	*N*	*P*	*P – PP*
7	*N*	*N*	*N*	*N*	*NP*	*N*
8	*NP*	*PP*	*N*	*NP*	*PP*	(?)[1]
9	*P*	*N*	*N*	*N*	*NP*	*N*
10	*PP*	*PP*	*NP*	*NP*	*P*	*P – PP*
11	*N*	*N*	*N*	*N*	*N*	*N*
12	*PP*	*PP*	*P*	*P*	*PP*	*PP*
13	*PP*	*PP*	*PP*	*P*	*PP*	*PP*
14	*N*	*N*	*N*	*N*	*NP*	*N*
15	*PP*	*PP*	*PP*	*P*	*PP*	*PP*
16	*NP*	*N*	*NP*	*NP*	*P*	*NP*
17	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*
18	*PP*	*P*	*P*	*N*	*NP*	*P – PP*
19	*PP*	*P*	*PP*	*PP*	*P*	*PP*
20	*N*	*N*	*N*	*N*	*N*	*N*
21	*PP*	*N*	*P*	*PP*	*PP*	*PP*
22	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*
23	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*
24	*N*	*N*	*N*	*N*	*N*	*N*
25	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*
26	*NP*	*N*	*NP*	*N*	*NP*	*NP*
27	*N*	*N*	*N*	*N*	*NP*	*N*
28	*PP*	*PP*	*PP*	*PP*	*PP*	*PP*
29	*N*	*P*	*NP*	*N*	*NP*	*N – NP*
30	*PP*	*PP*	*PP*	*P*	*PP*	*PP*

[1] It has been found impossible, owing to the wide variations recorded by the pathologists, to state the majority result for this serum. There is, in general, a tendency towards the positive side.

It is now possible to compare the results of the five pathologists. In the case of ten sera (33·33 per cent.) there is absolute agreement between the five. In six of these the result is *PP* and in four it is *N*. In the case of six more of the sera (20 per cent.) the only difference is between *P* and *PP*. Since this is not of great importance clinically it may be taken that in these six results the pathologists are also agreed. So there is agreement between all the pathologists as to the results being positive or negative in 53·33 per cent. of the cases. In the results of seven sera (23·33 per cent.) four agree with one another and the fifth differs. Three agree as to the results while two differ from the majority in the case of five sera (16·66 per cent.). Only in the case of two sera is there lack of agreement between any three pathologists.

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We may now consider the differences between these five pathologists' returns. Variations between *P* and *PP* are disregarded.

Table IV.

Pathologist	Serum No.	Report of pathologist	Majority report
A	9	*P*	*N*
B	1	*NP*	*PP*
	2	*N*	*PP*
	16	*N*	*NP*
	21	*N*	*PP*
	26	*N*	*NP*
	29	*P*	*N – NP*
C	8	*N*	*NP – PP*
	10	*NP*	*P – PP*
D	6	*N*	*P – PP*
	10	*NP*	*P – PP*
	18	*N*	*P – PP*
	26	*N*	*NP*
E	7	*NP*	*N*
	9	*NP*	*N*
	14	*NP*	*N*
	16	*P*	*NP*
	18	*NP*	*P – PP*
	27	*NP*	*N*

It should be noted that five of B's six divergences from the majority lie in swinging the result to the negative side and that *D*'s four are of exactly the same nature. *E*, on the other hand, makes the results of five sera more positive than they really are. It is probable that the antigens used by *B* and *D* are not sufficiently sensitive, while that of *E* is too sensitive for the amount of complement employed.

The errors may be divided into two classes, the serious and the moderate. In the first group are those in which the report is *N* while the majority are *P* or *PP*, and those in which the report is *P* or *PP* while the majority report is *N*. Such errors may cause the clinician to make mistakes either in the diagnosis or treatment of his cases.

Less serious errors are those of degree, *N* for *NP* and vice versa, *P* or *PP* for *NP* and *NP* for *P* or *PP*. These errors may deceive the clinician, but are less likely to do so than those in the first group.

Working on this basis, the results of each pathologist may be compared. The results have been tabulated in Table V.

Table V.

	A	*B*	*C*	*D*	*E*
Number of results agreeing with majority	29	24	28	26	24
Percentage of results which agree with majority (assumed to be correct)	96·66	80	93·33	86·66	80
Serious errors	1	2	1	2	0
Percentage of serious errors ...	3·33	6·66	3·33	6·66	0
Moderate errors	0	4	1	2	6
Percentage of moderate errors	0	13·33	3·33	6·66	20

The next question to consider is the reliability of each pathologist's method. There is no doubt that the first place goes to *A* and the second to *C*. There is also no doubt that *B* deserves the last place. The question whether *D* or *E* should be placed third is more difficult. *D* has only made four errors as against *E*'s six, but two of *D*'s are serious, while four of *E*'s errors are very slight, consisting in reporting negative as *NP* (in one, "a trace to 1"; in the second, "a trace"; in the third, " + 1"; and in the fourth, "a trace only"). It is difficult to decide between *D* and *E* but, on the whole, despite *E*'s greater number of errors, I am inclined to put *E*'s results as somewhat more reliable than *D*'s, chiefly on the ground of absence of gross errors in *E*'s results. The order of reliability is, therefore,

A
C
E
D
B

Considerable attention has been paid to the question of errors, but one must not fail to observe that even the least reliable pathologist has given results which agree in 80 per cent. of the specimens examined with those of the majority, which are taken to be the correct results.

In 15 sera the correct result was clearly strongly positive (*PP*). In all 75 tests of these 15 sera were made by the five pathologists and in only three cases (4 per cent.) were the results not definitely positive. These three errors were made by one pathologist (*B*). On the eight clearly negative sera, 40 tests were made and in five reports the results were not clear negatives. Of these five errors, four were made by one pathologist and consisted in reporting the results as *NP* ("a trace," etc.).

Errors were made chiefly in the case of sera which were weakly positive, that is, border-line cases in which it is always difficult to obtain uniform results.

One hundred and fifty examinations in all were done and correct results were returned in 131, that is, in 87 per cent. The serious errors only amounted to 4 per cent. of all examinations.

It may, therefore, be claimed that, in performing the Wassermann test, correct results are obtained in a greater percentage of the cases than in practically any other clinical test in existence.

Although the results of the control experiment here recorded are satisfactory, it would be desirable, if possible, to secure even greater uniformity. In order to do so, the following recommendations are made:

1. A uniform method should be adopted by all the pathologists working under the schemes of the Local Government Board in Ireland. My preference would be for that of Harrison which is now used by three of the pathologists.

2. The details of the method should be rigidly standardised, especially as regards the following: antigen, haemolysin, blood suspension, time and temperature of fixation.

As regards the first two, it would appear well to supply to each pathologist a uniform, tested antigen, and a carefully tested haemolytic serum. A supply could be issued every month or two, and so it could be made certain that the same batch was in use by each pathologist at the same time. In the report of the Medical Research Committee already referred to, this was recommended, but no steps have been taken to secure such a supply. It would be possible for the Board to undertake the manufacture and supply of these two articles. As regards the suspensions of blood cells used, I believe it to be of great importance to secure suspensions of uniform strength. A simple method of doing so has been described by me elsewhere[1].

3. A uniform method of reporting the results of the test should be adopted.

CONCLUSIONS.

A considerable degree of uniformity of results of the Wassermann test has been found among five pathologists.

Certain differences (chiefly in the case of weakly positive sera) have, however, been noted. Their main source lies in the use of different methods and not in any lack of personal ability or care on the part of the worker.

It is believed that the number of these could be greatly reduced by adopting a uniform method of performing the test and by standardising the details of the method.

The supply of a standard antigen and haemolysin to pathologists is recommended.

[1] Bigger. "The Standardisation of suspensions of Red Blood Cells for Wassermann Tests." *Lancet*, 1921, II. 1369.