

COMPLEMENT-FIXATION TECHNIQUE

III. THE WASSERMANN REACTION

BY

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In previous articles in this Journal (Orpwood Price and Wilkinson, 1947; Orpwood Price, 1949, 1950), the standardization of red blood cells, the titration of complement, and the titration of Wassermann antigen have already been described. It is now proposed to give a description of the test proper as employed at the Whitechapel Clinic and the V.D. Reference Laboratory.

The test proper consists of a screening procedure. Each serum has two tubes allotted to it. The front tube is the serum control tube and the back tube is the diagnostic tube. The contents of these tubes

in the case of both sera and cerebrospinal fluids are indicated in Table I.

Procedure

When these contents have been added, the racks containing the tubes are allowed to stand on the bench for 30 minutes and then placed in a water bath at a temperature of 37°C. for a further 30 minutes. At the end of this time the tubes are taken out of the water bath and 1 vol. sensitized red blood cells is added to each. The contents of the tubes are then well mixed by shaking the racks and replaced in the water bath, and by the end

TABLE I
CONTENTS OF TUBES FOR SCREENING


















Sera	1	2	3	Contents
Serum diagnostic tube				Serum $\frac{1}{4}$ vol. Saline 1 vol. Diagnostic dose of complement 1 vol. Antigen 1 vol. Sensitized RBC 1 vol.
Serum control tube				Serum $\frac{1}{3}$ vol. Saline 2 vols. Serum control dose of complement 1 vol. Sensitized RBC 1 vol.
Reading	Negative	Weakly positive	Positive	
Cerebrospinal Fluid	1	2	Contents	
CSF diagnostic tube			Neat cerebrospinal fluid 1 vol. Diagnostic dose of complement 1 vol. Antigen 1 vol. Sensitized RBC 1 vol.	
CSF control tube			Neat cerebrospinal fluid 1 vol. Saline 1 vol. Control dose of complement 1 vol. Sensitized RBC 1 vol.	
Reading	Negative	Weakly positive		

TABLE II
TUBES FOR QUANTITATIVE TESTS

Serum diluted 1 in 20 	Serum diluted 1 in 40 	Each tube contains : Appropriate dilution of serum 1 vol. Diagnostic dose of complement 1 vol. Antigen 1 vol.
Serum diluted 1 in 10 	Serum diluted 1 in 80 	
Serum diluted 1 in 5 	Serum diluted 1 in 160 	
Serum control diluted 1 in 5 		Serum control tube contains : Serum diluted 1 in 5 1 vol. Saline 1 vol. Serum control dose of complement 1 vol.

of 30 minutes all the serum reactions should have been read. Complete haemolysis in both tubes appertaining to any particular serum is read as a negative reaction. Partial haemolysis in the serum diagnostic tube with complete haemolysis in the serum control tube is read as a weakly positive reaction. No haemolysis in the serum diagnostic tube with complete haemolysis in the serum control tube is read as a positive reaction. No result can be given if the serum control tube of any particular serum fails to haemolyse completely. Any partially positive or completely positive sera are then put up for a quantitative test.

Quantitative Tests.—In these the technique is as follows. Each serum requires seven tubes, which are set up as shown in Table II.

As can be seen, the serum control tubes contain 1 vol. 1 in 5 dilution with normal saline of the serum to be tested, and doubling-up dilutions of serum with normal saline are made from the master dilution of 1 in 5 up to 1 in 160. Subsequently, the serum control tube contains 1 vol. control dose of complement and 1 vol. saline, whilst all the other tubes contain 1 vol. diagnostic dose of complement and 1 vol. antigen. The incubation and times are those of the test proper and the end-point is that tube which just fails to show complete sparkling haemolysis ; thus in the example above the




titre of serum would be reported as “ Positive, serum diluted 1 in 40 ”.

Cerebrospinal Fluid.—The set-up of this test is akin to that used in the case of sera. The contents of the tubes are similar, with the one exception of the cerebrospinal fluid itself. Neat cerebrospinal fluid is used in the control and in the first tube of the diagnostic row. The doubling-up dilutions are made with normal saline from the neat cerebrospinal fluid. The results are recorded in terms of “ Positive, neat ”, “ Positive, serum diluted 1 in 2, 1 in 4 ” and so on, depending upon which titre is relevant to the particular cerebrospinal fluid tested. The volume employed in the above technique is 0.11 ml. (Wyler, 1929).

In conclusion it should be stated that this technique is used for the complement-fixation test for gonorrhoea, and that in this case 1 vol. serum is used.

REFERENCES

Price, I. N. Orpwood (1949). *British Journal of Venereal Diseases*, **25**, 157.
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Wyler, E. J. (1929). *Spec. Rep. Ser. med. Res. Coun., London*, No. 129, p. 18–23.

Key to Tables {  = no haemolysis
 = partial haemolysis
 = complete haemolysis