A SIMPLE METHOD OF COLLECTING BLOOD FOR THE WASSERMANN TEST.¹

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THE usual procedure of obtaining blood for the Wassermann reaction by puncturing a vein of the arm with a 5 or 10 c.c. syringe, though satisfactory in itself, is not without its drawbacks. This is the case not infrequently to be found in a class of patients, especially neurasthenic and women subjects, where, for one reason or another, such operative measure is not feasible. To obviate this disadvantage and to enable a specimen to be conveniently taken at the patient's home, the following is a simple, yet reliable method, which I have tested in a number of cases with entirely satisfactory results, and one which can also be adopted in general.

Collection of blood.—For this method all that is necessary is a pricker and some pieces of filter-paper of the size of a sixpenny piece. I use Postlip Mills filter-paper, No. 633 Mill (ash = 0.00128 gr.), but any chemical-free absorbent paper of about that thickness may be employed. The thumb or the lobe of ear is pricked in the usual way, and specimens of blood are then taken with the papers, which, for the purpose, may be conveniently held in position with a pair of forceps. The size of the blood stain should be the size of the paper, that is, that of a sixpenny piece. I have found that each stain will contain roughly 0.08 c.c. blood, or about 0.04 c.c. serum. To ensure a uniform spread of film, it is best to allow a large drop of blood to accumulate before drawing it on to the centre of the paper. If the drop of blood is sufficiently large, the whole surface of the paper will almost immediately become covered with it by capillary action. A number of pieces of paper can be charged from successive drops. Care is to be taken that the films from the one case should all be spread, as far as possible, in a uniform fashion. It will augment the outflow of blood, if previous to the pricking or immediately afterwards, a bandage is tied round the finger and pressure exerted to its pulp. The number of such prepared papers sufficient for the test depends on the number of tubes to be used for each case. Some workers employ three as a routine, while others prefer more. As a rule, six is the maximum. When the required number, say six, of the papers have been so collected, they are ready to be dispatched to the Laboratory.

Method of testing.—No further treatment is required beyond allowing the papers to dry overnight, or in the incubator for a shorter time. For use they are lightly folded once or twice along one direction,

¹ Received December 17, 1917.

and, either before or after the other reagents (*i.e.*, antigen and complement) have been added, one, representing 1 dose, is dropped into each tube, which must finally contain sufficient fluid to completely cover the paper. The tubes are then shaken or inverted, and as in all other methods during the period of complement fixation in the incubator they are again shaken at least once, in this case advisedly after a quarter of an hour. Further steps are the same as when fluid serum is used, namely, at the end of one and a half hour's incubation a dose of ox or sheep blood corpuscles, previously sensitised with 3 M.H.D. of immune body, is added, and the reading is taken after a further incubation of an hour.

Remembering that each blood film represents 1 dose of the serum, any satisfactory method employing 1 dose in each tube may now be used. Some observers use the same amount of guinea-pig complement, usually 0.1 c.c. in each tube, but vary the doses of the patient's serum. This corresponds, to some extent, to the original Wassermann test. As the guinea-pig serum complement is a variable factor, a quantitative method is to be recommended. It consists in first titrating the hæmolytic dose of the complement; knowing this, graduated doses of the complement are added to a series of tubes containing the same amount of the suspected serum. One can thus determine the number of M.H.D. of complement fixed by each individual serum.

The method which I have adopted for the blood film test is briefly thus :

Preliminary stage.—The complement is diluted to 1 in 20, and to a series of tubes the following doses of this dilution are added : 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, 0.50 c.c. To another series of tubes containing the same graduated amounts of complement a dose of antigen is added. If the reaction against complement of the same stock antigen was previously known, the second row of tubes may be dispensed with. They are then titrated against 1 c.c. of a 2 per cent. solution of ox blood corpuscles, sensitised with 3 M.H.D. of immune body. Knowing the M.H.D. of complement by itself and in the presence of the antigen the next step is then proceeded with.

Final stage.—For each case, six tubes are put up in the manner as shown in Table I.

It is found that 2 doses of complement are usually absorbed by the blood film alone, so that if complete lysis takes place in tubes 1 and 2, which contain 3 and 4 M.H.D. of complement respectively, the dose so inhibited is taken as 2. Similarly, if complete lysis is observed in tube 2 but not in tube 1, the complement thus absorbed is 3 doses.

When, for any reason, it is desired to reduce the number of tubes in the experiment, three instead of six tubes may be used for each case, namely, tubes Nos. 2, 4, and 6, containing respectively 4, 6, and 8 M.H.D. of complement as indicated in the table. In this instance, the amount of complement deviated by the blood alone is taken as 3 doses if, as it usually does, complete lysis occurs in the control tube.

Interpretation of results.—The reaction is accepted as positive when 5 or more doses of complement are deviated in the presence of antigen,

in addition to the amount inhibited in the control tubes (Muir and Ritchie, 1913,¹ and Browning and Mackenzie, 1911²).

TABLE I.

Tubes	1.	2.	3.	4.	5.	6.
Complement (No. of M.H.D.)	3	4	5 +	6 +	7 +	8 +
Antigen	0	0	1 dose	1 dose	1 dose	1 dose
Blood film	1 dose	1 dose	1 dose	1 dose	1 dose	1 dose
Saline add up to (Incubate one and a half hour)	5 c.c.	5 c.c.	5 c.c.	5 c.c.	5 c.c.	5 c.c.
2 per cent. O.B.C. sensitised } with 3 M.H.D. of I.B. (Incubate one hour)	1 c.c.	1 c.c.	1 c.c.	1 c.c.	1 c.c.	1 c.c.
+ = plus the amount, if any, of complement deviated by the antigen alone. If the quantity so fixed is less than one-half M.H.D. it may be neglected.						

If less than three doses of complement are absorbed over the amount fixed by the controls, the result is negative.

Should the reading fall between these two limits the reaction may be regarded as doubtful.

When the test is carried out in patients who have undergone or are undergoing a course of anti-syphilitic treatment, it is absolutely necessary to record the results in so many doses of complement inhibited.

Control tests.—With this method a number of control tests have been made in the following ways:—

1. The serum from each case was divided into two portions. In one the serum was treated in the usual way by inactivating at 56° C. for half an hour. From the other untreated portion measured quantities of the serum were spread on a series of filter-papers and dried. Both preparations were tested for complement fixation at various periods after collection.

2. Blood films prepared from the finger in the manner described above, as well as the blood drawn from the arm vein of the same patient, were similarly tested. In all the cases examined, amounting in all to over fifty cases, the results obtained by the wet and the dry method were identical in general characters, and may be illustrated by the examples in the table given below.

It will be seen, then, that the substance reacting to antigen (so called immune body), for a given dose and at different stated intervals after collection, is less active in the wet inactivated serum than in serum inactivated by drying on paper. The results further show that the test by the blood film method is as delicate as that by the fluid

TABLE II.

	Number of M. H. D. of Complement deviated over that in the Controls.							
	18.	20.	22.	24.	26.	28.	30.	32.
Case 46.								
Wet serum inactivated by heat (dose, 0.05 c.c.)—								
2nd day after heating . .	-	-	+	+	+	+	+	+
9th " " . .	-	-	+	+	+	+	+	+
16th " " . .	+	+	+	+	+	+	+	+
Serum inactivated by drying on paper (dose, 0.05 c.c.)—								
2nd day after drying . .	-	-	-	-	-	-	-	+
9th " " . .	-	-	-	-	-	-	-	+
16th " " . .	-	-	-	-	-	-	+	+
	6.	7.	8.	9.	10.	11.	12.	13.
Case 26.								
Wet serum inactivated by heat (dose, 0.05 c.c.)—								
2nd day after heating . .	-	-	-	-	-	+	+	+
9th " " . .	-	-	-	-	+	+	+	+
16th " " . .	-	-	+	+	+	+	+	+
Serum inactivated by drying on paper (dose, 0.05 c.c.)—								
2nd day after drying . .	-	-	-	-	-	-	-	+
9th " " . .	-	-	-	-	-	-	+	+
16th " " . .	-	-	-	-	-	+	+	+
Case 35.								
Wet serum inactivated by heat (dose, 0.05 c.c.)—								
3rd day after heating . .	-	-	-	-	-	+	+	+
10th " " . .	-	-	-	-	+	+	+	+
17th " " . .	-	-	-	+	+	+	+	+
Blood film inactivated by drying (sixpenny size)—								
3rd day after drying . .	-	-	-	-	-	+	+	+
10th " " . .	-	-	-	-	-	+	+	+
17th " " . .	-	-	-	-	+	+	+	+
+ = Hæmolysis. - = No hæmolysis.								

serum, when the latter is used in doses of 0.05 c.c.; this being the optimum amount, according to Browning, to elicit the reaction.

In all the negative cases examined the results arrived at were the same in both methods.

It may be said that pieces of filter-paper of the size used in my investigation do not by themselves absorb complement.

CONCLUSIONS.

By way of conclusion the following points may be mentioned:—

1. The dry method is simple in manipulation and reliable, and a

specimen of blood is as conveniently taken in the patient's home as in the hospital.

2. While suitable for all cases, with the possible exception, perhaps, of such cases as scarlet fever and typhoid fever, it is especially commendable to a class of neurasthenic patients and women, thus allowing a larger number of "suspects" to be submitted to the test.

3. Although the blood in this dry condition preserves its activity for a long time, it is advisable that, whenever possible, the films should be used within a week of collection.

4. It is shown above that, for the same dose, the specific serum when inactivated by drying on paper is capable of deviating a larger amount of complement in the presence of antigen, in addition to that absorbed in the controls, often to the extent of from 2 to 10 or more M.H.D., than the serum inactivated by heat. It would appear as if heat may have an unfavourable effect on the substance in the serum which combines with antigen, and therefore it is preferable that, in rendering the human complement ineffective, drying instead of heat should be used; this can readily be done, if the blood is taken in the ordinary way from the vein, by measuring the necessary doses of the separated serum on pieces of absorbent paper and drying in the manner described.

5. With the issue of the recent Venereal Diseases Orders, there will be an increasing claim on the Wassermann test as a means of diagnosis. It is therefore desirable that a fixed standard of interpretation of the reaction should be set up and adopted, so as to enable the findings to be recorded in a comparable manner. As it is just now, different workers have their own keynote in defining their results. Some would take it as positive with an inhibition of 2 doses of complement, in addition to the controls, while others would only accept a positive result when the deviation of complement is 5 or more doses. Such divergence of interpretation can only lead to confusion, and will tend to undermine the value of the test.

I have much pleasure in thanking Miss FitzGerald for courtesy in providing me with some of the material, and Professor Ritchie for other favours.

REFERENCES.

1. MUIR AND RITCHIE . . "Manual of Bacteriology," 6th ed., London, 1913.
2. BROWNING AND MACKENZIE "Diagnosis and Treatment of Syphilis," London, 1911.