CHOICE AND PREPARATION OF BLOOD-GROUPING SERA FOR EMERGENCY PURPOSES

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During the past few weeks we have been confronted with the problem of supplying relatively large quantities of human typing sera at a few hours' notice, and the purpose of this paper is to record the methods we employed, in the hope that they may be of help to others faced with the same situation.

The selection of the typed volunteers constituted our first problem, and this was accomplished with the aid of a small quantity of blood-grouping sera* of Group II (Moss)—that is, international classification A; and Group III (Moss)—that is, international classification B. With these a number of human volunteers were duly typed. (For the remainder of this paper we have adopted the Moss classification.) From these individuals four of Group II and four of Group III were chosen for tests, the object of the experiments being to find out which of them could provide the most active sera for standard typing purposes. In order to obtain this information it was decided to set up a series of increasing dilutions of each individual's serum, to which was added a suspension of red blood cells. In this way it was possible, first, to determine which of the sera produced clumping in the highest dilution, and, secondly, to ascertain the character and nature of the clumping produced. The results of our experiments showed that the serum possessing the highest end-titre of agglutinins was not necessarily the serum of choice for typing purposes, since we observed that the qualitative character of the reaction—for example, the size and appearance of the clumps as seen by the naked eye—was a better criterion of the value of sera for typing work than the end-titre at which the reaction occurred. We wish to point out that some sera give agglutination reactions that are easily visible to the naked eye, whereas with others such reactions are not so marked and the time taken for agglutination is longer. It is desirable to add

a preservative to typing serum, and of the two most suitable reagents, phenol and glycerol, we decided to use phenol in order to avoid excessive dilution of the serum. For a comprehensive account of human blood groups the reader is referred to the monograph of Dawood Matta (1937). Details of the experimental methods employed by us are as follows.

Collection of Blood

Ten c.cm. of blood was removed from each of four Group II donors, was allowed to clot overnight in the ice-chest, and was separated from the sides of the test tube with a platinum wire and centrifuged; the serum was then separated. From one of this same group a further 10 c.cm. of blood was withdrawn, added to 10 c.cm. of 3.2 per cent. sodium citrate solution, centrifuged, the deposited corpuscles washed thrice in physiological saline, and 100 c.cm. of a 1 per cent. suspension in saline of these cells prepared. There was now available (1) about 3 c.cm. of serum from four different Group II donors, and (2) 100 c.cm. of a 1 per cent. saline suspension of washed Group II corpuscles. Exactly the same procedure was adopted with regard to the four Group III donors, so that four specimens of Group III serum—that is, containing agglutinin α —were available, together with one specimen of Group III corpuscles—that is, containing agglutigen B.

Technique of Titration Test

For the testing of each serum eleven Wassermann tubes were placed in a rack and 0.4 c.cm. of saline was added to tubes Nos. 2 to 11 inclusive. To the first and second tubes 0.4 c.cm. of serum (from the first donor to be tested) was added. From tube No. 2, 0.4 c.cm. of the diluted serum was transferred to tube No. 3, and from this tube the process was repeated until tube No. 10 was reached, from which 0.4 c.cm. was discarded. The serum dilutions of No. 1 donor were thus as follows: 0.4 c.cm. of undiluted serum in the first tube, 0.4 c.cm. of 1:2 serum in tube No. 2, 1:4 in No. 3, and so on with the highest dilution of 1:512 in tube No. 10. Tube No. 11 contained only 0.4 c.cm. of saline and constituted the control. Red blood corpuscles of Group III were next added (0.4 c.cm. of a 1 per cent. suspension in saline to each tube), so that each dilution of serum became doubled, and thus tube No. 1 contained a final dilution of serum diluted 1:2, and tube No. 10 a dilution of 1:1024. The control tube now consisted of a mixture of normal saline and red blood cells. The test tubes were then incubated at 37° C. for one hour; at the end of that time a preliminary examination of the tubes was made with the aid of a hand lens, following which the tubes were allowed to stand overnight on the

* Burroughs Wellcome and Co., London.

Final Serum Dilution (against Group III Corpuscles)

	1	2	3	4	5	6	7	8	9	10	11
Group II Serum	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	Control
Donor 1	+++	+++	+++	+++	+++	++	—	—	—	—	—
„ 2	+++	+++	+++	++	+	—	—	—	—	—	—
„ 3	++++	+++	+++	+++	+++	++	+	—	—	—	—
„ 4	+++	+++	+++	+++	+++	++	+	±	—	—	—

Final Serum Dilution (against Group II Corpuscles)

	1	2	3	4	5	6	7	8	9	10	11
Group III Serum	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	Control
Donor 5	+++	+++	+++	+++	++	+	+	±	±	—	—
„ 6	++++	+++	+++	+++	++	+	+	±	—	—	—
„ 7	+++	+++	+++	+++	+++	++	+	±	—	—	—
„ 8	+++	++	++	++	++	++	++	+	±	—	—

NOTE.—The sign + indicates agglutination.

laboratory bench at room temperature, being subjected to a final reading in the morning (after eighteen hours).

This method of testing serum (No. 1) for haemagglutinating properties was repeated in the case of the other three sera (of Group II). In actual practice it was our procedure to set up all the serum dilutions simultaneously, add corpuscles of Group III to the four Group II sera and corpuscles of Group II to the four Group III sera, incubate the test tubes together, and interpret all the results, first at the completion of one hour and later at the end of eighteen hours. The table indicates the results obtained.

From the foregoing table the following definite fact can be observed—namely, that each of the diluted sera tested gave approximately the same end-agglutination titre (approximately 1:128 for serum of Group III Moss). We wish to point out that human sera possess different agglutinin end-titres, but in the present work the ones used happened to have approximately equal end-titres; also, in each group variations are found in the degree of agglutination given by different corpuscles with the same serum. It was seen, too, that each of the four sera showed a dilution at which optimum agglutination occurred. As already stated, in addition to these quantitative observations there were definite qualitative differences in the nature and size of the clumping which enabled us to select certain of these sera as being the most suitable for the purpose of blood grouping.

Storage of Serum

Having chosen the two most suitable donors from each of Groups II and III, half to three-quarters of a pint of blood was withdrawn from each donor into sterile pint bottles and allowed to stand overnight in the ice-chest. During this preliminary storage the absorption of any "cold" agglutinins takes place. The separated serum was transferred by means of sterile pipettes into sterile 100 c.cm. bottles. In order to maintain sterility phenol (5 per cent. aqueous solution) was then added to the serum so that the phenol concentration was 0.5 per cent. For distribution purposes the phenolized serum was transferred in 1 c.cm. quantities to sterile phials fitted with rubber bungs, which were afterwards waxed.

Use of Desiccated Serum

An alternative method for the preservation of typing sera which we used was that employed earlier by Craigie (1931) and Craigie and Tulloch (1931) for the preservation of sera by exsiccation *in vacuo* from the frozen state. Using this method, it is possible to dry rapidly in a desiccator over CaCl_2 large quantities of serum. The final product, which assumes the character of a dry brown flaky powder, can be stored in the ice-chest for an indefinite length of time without fear of contamination or significant loss of agglutinating properties. On account of its hygroscopic nature the container should be sealed or well stoppered. The only disadvantage of the use of dried serum is that larger quantities are required than of the fluid serum; but this disadvantage is offset by the fact that desiccated serum is easily distributed and handled, and, in any case, the actual amount of dried serum needed for a typing test is exceedingly small.

When it is desired to make a quantity of serum from dried material it is necessary to reconstitute the dried serum to the original bulk by the addition of sterile distilled water; thus if 5 c.cm. of serum were desiccated to a dry powder, 5 c.cm. of water should be added to it in order to restore its original consistency.

Technique of Typing Test

For typing human serum we have employed the standard macroscopic procedure of the London Blood Transfusion Service. Briefly, this was performed by placing two large drops of typing sera of Groups II and III respectively on a glass plate, to each of which was added *one small* drop of blood obtained from a finger-prick, care being taken to see that only enough blood was added to colour the mixture light pink, but not deep red. After thoroughly mixing the blood and serum by agitating the plate, the results were usually evident in one to two minutes. The use of a hand lens was occasionally of assistance in interpreting the phenomena, but, in general, neither a hand lens nor a microscope was required.

Technique of Test employing Dried Serum

A few small flakes (one bacteriological loopful) of dried serum of each of Groups II and III were placed on a glass plate; a large drop of water was added to each and the serum allowed to dissolve by gentle mixing. The subsequent stages of the test were performed in the same manner as in the case of fluid serum.

Conclusions and Summary

From our observations we conclude that the selection of suitable blood intended for the purpose of providing standard human blood-grouping sera should be assessed in two ways: (1) according to the end-titre of agglutinins; (2) by considering also the character and size of the clumps produced after a given length of time.

The use of desiccated typing sera is described and its merits for war-time work emphasized.

We wish to record our thanks to the Edinburgh Blood Transfusion Emergency Service for help in withdrawing the blood from the donors selected by us, and to Professor C. H. Browning for his interest and valuable advice.

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A quarterly court of the directors of the Society for Relief of Widows and Orphans of Medical Men was held on October 11, with Mr. V. Warren Low, president, in the chair. The deaths of a member and two of the widows in receipt of grants were reported. One of the widows had been in receipt of grants for the past thirty-four years and had received £3,500 from the society. Her husband, who was elected a member in 1868, had paid £52 10s. in subscriptions. The audited accounts for the half-year ended June 30 were presented and passed. During that period £2,242 10s. had been distributed among the sixty-two widows and four orphans on the books of the society. A widow of a member applied for relief, and was granted £60 yearly. The grants in January, 1940, will total £2,152. Owing to the increase in income tax the directors will not be able to make a Christmas present this year although the amount of income tax is returned, as the reclaim is not paid until about the middle of April in each year. Membership of the society is open to any registered medical man who at the time of his election is resident within a twenty-mile radius of Charing Cross. Relief is granted only to widows and orphans of deceased members, and they must be in necessitous circumstances. Full particulars may be obtained from the secretary of the society, 11, Chandos Street, Cavendish Square, W.1.