Nov. 13, 1956 KNUD-ERIK H. ELDON 2,770,572

BLOOD GROUPING CARD

Filed July 24, 1952

Fig. 1.

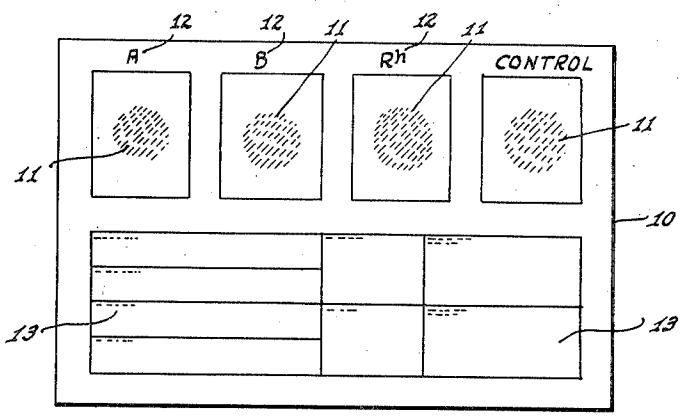


Fig. 2.

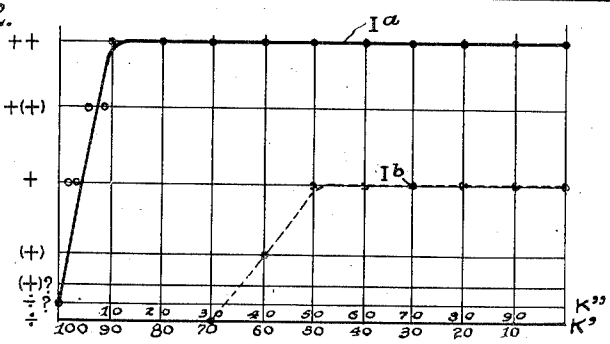


Fig. 3.

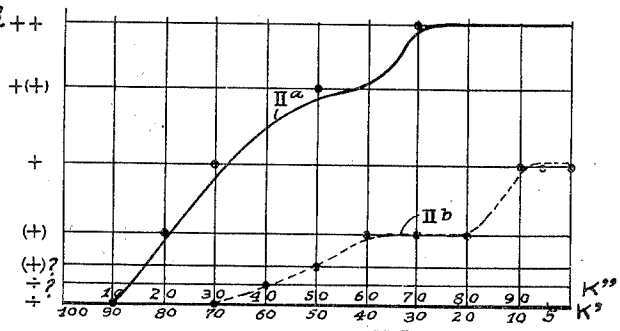
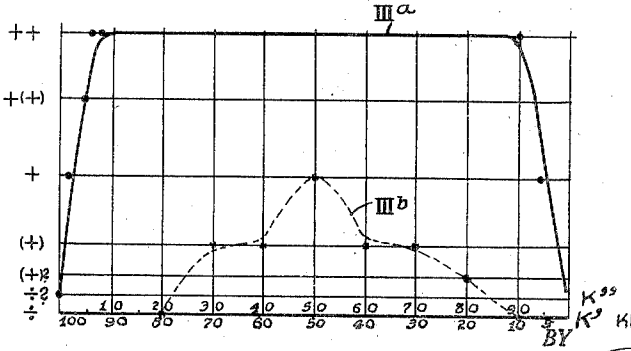


Fig. 4.



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BLOOD GROUPING CARD

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10 Claims. (Cl. 167—84.5)

This invention relates to means for blood grouping. The blood groups of practical importance in humans are the A, B, AB, O and Rh groups.

In testing for A and B anti-factors the method hitherto generally used consists in mixing the test serum in question—i. e., serum containing A-antibody or B-antibody—with a saline suspension of red cells from the blood of the patients. The mixture can be produced upon a glass slide or in a small test tube; and after the glass slide has been tilted back and forth for ten minutes or the tube has remained standing for two hours the reaction is read.

This method of grouping with respect to the A, B, AB, or O-type is deficient in several ways. Thus, incorrect results will be obtained if the test sera for the A and B grouping respectively have been inadvertently interchanged or if the blood to be grouped has been inadvertently changed for the blood of another patient. Moreover, the red cells in the blood of some humans possess the property of agglutinating in any serum independent of the contents of specific antibodies in said serum, and consequently when testing such blood by the common method, the tests will be read as AB which in the most cases will be incorrect, since the AB group is rather rare. On the other hand, the said characteristic of the red cells, called panagglutination, is still more rare. Further, in the case of grouping new-born children by means of blood from the umbilical cord the method may for other reasons give rise to a similar false AB-reading.

Another method of determining the groupings A, B, AB and O has been described by G. F. Wagner in "Der Deutsche Militärarzt" of March 1941. It consists in using for the determination a cardboard upon which spaced drops of each of three test sera respectively containing anti-A bodies, anti-B bodies, and both anti-A and anti-B bodies have been evaporated, thus forming three test areas on the surface of the card. These areas are marked appropriately to indicate the kind of test for which each of them is adapted. When using the cardboard a drop of saline solution and a drop of blood of the patient are spread over each of the three areas and mixed with the test serum thereof. The cardboard is then tilted back and forth towards all directions alternately for a few minutes and the result of the tests is read. Agglutination of the blood in the areas containing anti-A and anti-B bodies indicates presence of A and B respectively, whereas absence of agglutination in any area, including the AB-area, indicates grouping O.

This method like the first mentioned method has the disadvantage of reacting on panagglutination as if the blood were of the type AB. Furthermore, it gives rise to unspecific reactions, very similar to the positive reactions, as a result of the rouleaux formation. Addition of a colloidal lecithin-saline solution has been proposed by Wagner to prevent this drawback, but such addition often inhibits also the specific agglutinations. Accordingly, this method has found no practical use, in spite of its

obvious advantages as to saving of time and diminished risk of mistakes in identity.

While the determination of the A, B, AB and O-groupings can be made over a range of ordinary temperatures, in the determination of the Rh-grouping a temperature of 37° C. should be used in the methods hitherto normally used. When carrying out these determinations in large laboratories a saline suspension of the red cells of the patient is usually mixed in a small test tube with anti-Rh serum, after which the tube is left to stand for one and a half to two hours at 37° C., when the reaction can be read. For carrying out this method regular supplies of anti-Rh sera capable of reacting in a saline medium are required. Such supplies cannot be had regularly in smaller laboratories which must, therefore, content themselves with a method in which the sera used are of the incomplete type reacting only on cells suspended in a liquid containing conglutinin or conglutinin substitutes. The method used by such smaller laboratories is usually the following: An incomplete anti-Rh serum of the said kind is mixed with red cells of the patient suspended in their own serum. In many cases the mixture is prepared on a microscope slide which is then placed upon a heating box maintained at about 37° C.; and reading can be safely carried out after three minutes. It is necessary to use coagulated blood for the examination, since, when uncoagulated blood is used, falsely positive results may occur as a consequence of the sedimentation constant of the blood being slightly higher than normal. Even when this security measure is employed, falsely positive results may occur in blood having an exceptionally high sedimentation constant, such as exists, for instance, in certain feverous conditions, certain kinds of cancer, certain blood diseases or in consequence of the patient being in the latter part of the period of pregnancy. In the case of panagglutinability the results will be falsely positive, whereas blood of new-born children strongly influenced by Rh-antibodies from the mother may give rise to falsely negative results.

Finally, all the methods mentioned above except the cardboard method of Wagner possess the disadvantage arising from the possibility of errors in identifying the source of the blood tested. In the cardboard method, where blood from the ear of the patient can be transferred directly to the surface of a card filled in with identifying data in the presence of the patient, the risk of misidentification of specimens is minimized.

One object of my present invention is, therefore, to provide a blood-grouping test which can be carried out at the side of the patient, which will not require a skilled technician, and the accuracy of whose results will not be interferred with by pseudo-agglutination.

Another object of my invention is to provide means by which all test sera required for the grouping, including anti-Rh serum are placed in separated areas or fields safely marked on a single supporting sheet, all being adapted to show the correct result when subjected to identical treatment.

Still another object of my invention is to provide a supporting sheet upon which the test sera are safely carried in a dry state until using them, and upon which the reaction mixture after the result has been read will dry to show just as clearly as in its moist state whether the reaction has been positive or negative, which requires that even on completion of the drying no pseudo-agglutination takes place.

An additional object of the invention is to provide a blood-grouping test which can be carried out over a wide temperature range and which will disclose cases of pseudo-agglutination which might otherwise result in an erroneout typing of blood as belonging to the AB group.

In carrying out the invention in its preferred form, 1

provide a supporting sheet bearing on different portions of its surface dried specimens of different test sera, one of which is an indifferent serum. Each surface-portion of the card bears appropriate indicia identifying the character of the dried serum it bears. Before application to the sheet, the several sera are mixed in uniform proportions with a substance or a mixture of substances of the class consisting of conglutinins and conglutinin substitutes, the added substance or substances being the same and being employed in the same proportion for all sera on any one card. Further, the several sera used on each card are standardized by the addition of indifferent serum so that when the test is later performed they will yield the reaction for which they are respectively specific. This makes it possible to include a test for Rh-factor in the blood cells on line with the other tests, and carry out all tests at any temperature between the freezing temperature of water, and say, 50° C., since incomplete anti-Rh specific sera has been found able to react with Rh-positive blood cells under these conditions.

With these and further objects and purposes in mind which will appear from the following, I will now describe a preferred embodiment of my invention, it being understood that the same is not limited to the said embodiment but only by the appended claims.

The means used according to my invention in all cases comprise a supporting sheet upon which a series of portions of test sera diluted in a suitable manner to be more closely described in the following are evaporated so as to cover limited areas of the surface of the supporting sheet, said areas being identified in accordance with the kind of serum contained therein by marking in print or otherwise.

In the accompanying drawing:

Fig. 1 illustrates one form of a suitable sheet or card bearing the dried test sera.

Figs. 2 to 4 are graphs illustrating results obtained with various different types of test sera.

In Fig. 1, I show a sheet or card **10** bearing spots **11** of dried test sera, each identified by appropriate indicia **12**. The card **10** may also bear appropriate spaces **13** for insertion of data such as the name of the patient, the date of the test, the test-result, etc.

The sheet **10** as shown is preferably of rectangular or other regular or simple form adapted to be conveniently stored in card files and the like or to be enclosed and sent in envelopes or as a postcard. It is preferably of the thickness of ordinary cardboard or strong paper or the like adapted to keep reasonably flat when used. Preferred thicknesses are in the range of 0.1–3 mms. depending on the material, the thinner materials being only suitable in case of very stiff materials such as metal or when they are used in connection with a stiffening frame or a stiff support to which they are fastened during the part of the reaction which involves the use of moisture.

The kind of sheet-material is otherwise unimportant except for the purposes of portability, stiffness and convenience in storing; and so long as reasonable requirements are fulfilled in these respects and the sheet is capable of keeping flat, with or without auxiliary means, during the part of the reaction where moisture is used, any thickness will do. In the case of cardboard, for instance, a thickness of 0.2–0.7 mm. will do.

The size of the sheet must be such as to provide space for the desired number of testing areas **11** and for identification notes in the spaces **13**. Since in most cases a test-area need not exceed 500 mm.^{2} and the number of such areas may be reduced to 4 or less, although it can be 8, 10 or more, card sizes of 8–15 by 5–10 cms. will be well suited for all ordinary purposes.

While the material of the supporting plate is mainly a practical and economical question, the nature and material of its surface or the part of the surface on which the portions of the test sera are placed and the reactions are to be carried out as described below, is of some importance so far as concerns such qualities as its smoothness, chemical and biological inertness, and its capability of maintaining and being moistened evenly by serum and water.

The smoothness of the surface must be of such a degree that the rather thin layer of moisture involved in the reaction will adequately cover any grain present in the surface, as otherwise a grainy appearance of the moist and dried surface covered by the reaction mixture will result, which would tend to interfere with accurate reading of the test results. An idea of the degree of smoothness required can be had by calculating the depth of the layer of liquid involved in each area during the reaction. Thus, for instance, each test area may be 300 mm.^{2} and the amount of liquid may be 10 mm.^{3} of blood and 50 mm.^{3} water making 60 mm.^{3} which corresponds to a depth of about $\frac{1}{5}$ mm. Consequently, in this case the roughness should not exceed $\frac{1}{5}$ mm. very much and is preferably far less.

As to the chemical and biological indifference of the material forming the surface parts of the supporting sheet it is quiet clear that the material should not give off substances reacting with the constituents of the sera and should not be soluble in water or capable of absorbing the constituents of the sera. Furthermore, it should not give off appreciable proportions of substances such as acids (c. g. acetic acid), aldehydes, alkalies and salts.

As to the capability of the surface of maintaining serum and water and of being moistened evenly thereby the points of practical importance are particularly that it must not to any substantial degree be porous or penetratable by water so that the liquid will be sucked into the pores of the material, and it must form a contact angle with water and solution of serum which is not so small that the liquid shows a great tendency to run off the surface when the same is tilted to, say, 45°.

I have found that a surface layer consisting of a film of regenerated cellulose, cellophane, particularly the brand brought on the market under the trade name of "Industriatape," is very well adapted for the purpose. A surface of hard paper glued in the paper mass by a vegetable glue such as resin, and glued on the surface by an animal glue such as recommended by Wagner is also suitable for the purpose. Furthermore, chemically indifferent metals such as gold have been found suited. A few lacquers or plastics have been found reasonably suitable, such as cellulose acetate, polyvinyl chloride and polymethyl acrylates. The metals, lacquers and plastics, however, although the reaction will be correctly read upon them when judging from the moist reaction mixture, are not satisfactory for surface layers in the case where it is desired that the reaction shall be equally well readable upon the dried-out reaction mixture, since rouleaux-formation tends to occur during evaporation to dryness.

The surface layer may also consist of a layer of hardened gelatine, as in photographic paper when fixed and washed. Since such gelatine layer is to some extent penetrable to water, as is also regenerated cellulose, I prefer, in both cases, to paste the surface layer to the support by means of a water-resistant adhesive, or to place between said layer and said support a water-resistant intermediate layer, for instance lacquer or metal foil, in order to prevent crinkling of the support.

The supporting sheets may consist themselves of another material than the surface parts carrying the test sera, for instance of paper, cardboard, Celluloid or plastics. If the sheet is to comprise a sera-bearing facing on a stiffening back, the facing need extend only over the area occupied by the test-sera **11**.

The sera used are the sera usually employed by the hospitals and laboratories carrying out blood grouping. They are commonly human sera produced from persons who spontaneously contain the antibody in question in their sera or who are immunized for that purpose. The blood recovered from the animal or person in question is

coagulated and the serum is removed from the coagulate and centrifuged if desired. The serum thus recovered is then subjected to the usual treatments to make it suitable as a reagent in blood grouping. The only treatment which is generally necessary is a heat treatment carried out by heating the serum to about 56° C. for about 20 minutes to destroy the complement which otherwise might cause hemolysis instead of agglutination. In the case of anti-Rh sera a further treatment will be necessary to remove undesired anti-A and anti-B antibodies by adsorption on suitable materials, such as the corresponding blood cells.

The sera recovered from individual donors is usually of varying strength as to the antibody in question. In carrying out my invention sera the strength of which exceeds the strength necessary to show a clear unmistakable agglutination reaction are required because dilution of the serum with conglutinin or conglutinin substitutes is necessary for several purposes, one being to avoid the occurrence of unspecific reactions due to rouleaux-formation, another being to maintain the concentration of conglutinin or conglutinin substitutes for the working of incomplete anti-Rh sera, and a third being to accelerate agglutination if so desired, all as explained below. The extent to which the sera are to be diluted for these purposes will be more closely specified below. It is, however, possible to obtain sera which will stand dilution to an extent exceeding that necessary for the said purposes, and I prefer standardizing and balancing such sera. For this purpose I mix them with indifferent serum or with sera which show the same specific reaction but are weaker than desired. In this manner the strong sera are utilized in a more economical way, and at the same time it is possible thereby to equalize the positive reactions appearing on one and the same support so that they will have nearly the same appearance and appear approximately after the same time of reaction.

In order to explain more fully the influence and purpose of the dilution of the sera with conglutinin or conglutinin substitutes reference is made to Figs. 2 to 4 showing a number of curves. The curves show the intensity of specific and unspecific reactions when the anti-Rh test serum employed is mixed with certain specific constituents k' and k'' in the relative proportion indicated, the constituents k' and k'' in each case falling in the class of substances consisting of conglutinins and conglutinin substitutes. The curves I*a*, II*a*, and III*a* show the intensity of the specific reactions, whereas the curves I*b*, II*b*, and III*b* show the intensity of the unspecific reactions. The intensities are estimated from the appearance of the reaction mixtures after three minutes on a card produced in accordance with my invention. The curves relate to the following mixtures:

I*a* and I*b* relate to a mixture in which k' is a 6% solution of dextrane and k'' is indifferent blood serum, which mixture contains anti-Rh serum in the ratio of 1:1000.

II*a* and II*b* relate to a mixture in which k' is a 20% solution of serum albumin, and k'' is indifferent blood serum, the mixture containing anti-Rh serum in the ratio 1:1000, and

III*a* and III*b* relate to a mixture in which k' is a 6% solution of dextrane and k'' is a 20% solution of serum albumin, the mixture containing anti-Rh serum in the ratio 1:1000.

The intensity of the unspecific reactions indicated by the curves I*b*, II*b* and III*b* are estimated from the behavior of mixtures to which blood giving no Rh-reaction is added, whereas the intensities of the specific reactions indicated by curves I*a*, II*a* and III*a* are estimated from reactions in which blood containing Rh positive cells is involved.

It will be seen that the specific reactions for the greater part of the mixtures are stronger in the cases I and III, where dextrane is involved, than in case II, where no dextrane is involved. Moreover, it appears from curves I*b* and II*b* that in the case, where 100 percent of the component k'' is present—i. e., when the only diluent of the specific serum is an indifferent serum—there will be a strong unspecific reaction. When, however, somewhat more than half the indifferent serum is replaced by the dextrane solution (curve I*b*) the unspecific reactions will be less pronounced, and when the ratio of dextrane to serum exceeds 4:1 there will be no unspecific reaction at all. For the purpose of considering the influence of serum on unspecific reaction it is of course immaterial whether the serum is specific or indifferent. This result, therefore, shows that dilution with dextrane will prevent unspecific reactions in all cases where the ratio exceeds 4:1, irrespective whether the specific serum has been standardized or equalized with indifferent serum or not.

As to albumin, curve II*b* shows that the unspecific reactions can also be avoided by replacing serum by this substance. In this case the range of dilution ratio within which significant unspecific reactions can be prevented will be even greater than in the case of the dextrane, but the intensity of the specific reactions (curve II*b*) within the range where unspecific reactions are avoided will be less. In the case of mixtures of dextrane and albumin solutions (curve III*b*) it is also possible to prevent unspecific reactions by using a diluting agent in which the proportion of albumin is high.

Serum and/or plasma itself contains conglutinin. Where, as in Figs. 2 and 3, a mixture to be dried on the card contains conglutinin provided by the serum (indifferent, specific or both) and also contains a conglutinin substitute, the ratio of the serum to the conglutinin substitute will generally lie in the range from 1% to 20% calculated as set forth below. In some cases it may be higher, for instance in the case of albumin. Preferably, I use the ratio of 1:9.

Where, as in Fig. 4, a test serum strong enough to stand a dilution of 1:1000 is diluted in that ratio with a mixture of two conglutinin substitutes, viz. dextrane and albumin, the conglutinin content of the mixture is insignificant (because of the low serum content), and the opposite ends of each of curves III*a* and III*b* are closely similar. It will be noted from Fig. 4 that in mixtures where the ratio of one conglutinin substitute to the other is from 1% to 20%, again calculated as set forth below, the specific reactions are markedly enhanced in intensity while the intensity of the non-specific reactions undergoes little if any enhancement.

As conglutinin substitutes may be used such substances falling within the definition given by Wiener in his paper: "Rh-Glossary" printed in "The Laboratory Digest," St. Louis, Mo., November 1950, provided that solutions thereof with sera are, after drying, readily re-dissolvable in cold water, for instance albumin solutions, gum acacia solutions, solutions of some high-molecular alcohols or their derivatives or solutions of some high molecular sugars. I prefer, however, to use 6% solutions of dextrane, particularly of the brand on the market under the name of "Macrodex," because such solutions in the presence of serum (specific or indifferent) or albumin highly accelerate the rate of reaction of the agglutination process as shown by Figures 2 and 4.

The concentration in which the conglutinin or conglutinin substitutes are used for preparing the diluted sera to be placed in the separate areas on the supporting sheet depends on the particular conglutinin or conglutinin substitute. For instance I may use a 6 percent solution of dextrane, a 20 percent solution of albumin, a 1 percent solution of polyvinyl pyrrolidine or a ½ percent solution of gum arabic. Solutions of such respective strengths have a viscosity approximately similar to that of blood serum, and a colloid-osmotic pressure to suit blood corpuscles.

In the present specification, when speaking of the ratio of serum to conglutinin substitutes or of one conglutinin substitute to another, such statements are based upon volumes of serum and volumes of solutions of conglutinin or conglutinin substitute in the concentrations in which they should be used to make the desired reaction mixture.

Among the conglutinin and conglutinin substitutes the

substances which have been used for infusion in the human blood vessels are generally preferred as far as they come within the definition of Wiener.

Other substances may also be added to the sera to be placed on the separate area of the support **10**, such as solutions of salts to control the osmotic pressure. I prefer to use such salts, for instance sodium chloride, in concentrations in which they form solutions isotonic or slightly hypertonic with blood, for instance 0.9 percent or more of sodium chloride.

I have found the most suitable salt concentration in the reaction mixture to be about 2 percent. In order to be able to use tap water or distilled water for re-dissolving the serum-conglutinin or serum-conglutinin substitute mixture on the card, the salt must be incorporated with the mixture of serum and conglutinin or conglutinin substitute before drying. Serum will always contain about 0.9 percent, and conglutinin or conglutinin substitutes are frequently prepared with a content of 0.9 percent of salt. However, the increase of the salt concentration to about 2 percent accelerates the specified reaction very considerably without producing unspecific reactions. On the other hand salt concentratons as high as 3 percent should be avoided, since this would substantially inhibit the agglutination. This also applies if, in the testing, a suspension of blood corpuscles is used instead of blood. Other examples of suitable admixtures are coagulation inhibiting substances, such as sodium citrate or sodium oxalate or heparin, preferably from other sources than the ox. If such additions are used the proportion may preferably be the same as used for approximately 5–40 mm.^{3} of full blood. In the case of heparin, for instance, one part of heparin solution (5 percent) may be used to 3000 parts of the mixture of serum and conglutinin or conglutinin substitute.

In the preferred form the sheet has only four areas covered by dried serum. The first area carries a mixture of anti-A serum (standardized to stand dilution in the ratio 1–10) and Macrodex, a second area carries a dried mixture of anti-B serum (standardized in the same manner) and Macrodex, a third area carries a dried mixture of anti-rhesus serum (which may be of the incomplete type which will not react in saline solution of blood cells but only with solutions containing conglutinin or conglutinin substitutes, which serum is again standardized in the same manner) and Macrodex and the fourth area carries indifferent serum mixed with Macrodex.

In all four areas the ratio between serum (standardized or indifferent) and Macrodex (6%) is 1:10.

Macrodex is the solution of a dextrane hydrolysate produced by the manufacturer in the concentrations in which it is adapted for infusion in the blood system. In place of Macrodex the other known conglutinins or conglutinin substitutes with which serum can be diluted (i. e., different from serum), can be used in the concentration in which they are employed when used for infusion. The amount of dilution stated above is calculated subject to the condition that the conglutinin or conglutinin substitute is used in this concentration, but of course it is not necessary that it is really used in such concentration, since the serum when applied to the supporting sheet is to be evaporated. The real concentration used is, therefore, only a practical question.

As a result of the great amount of conglutinin or conglutinin substitutes present the Rh-reaction will safely take place within 3 minutes and all portions of serum will remain without unspecific reaction (rouleaux-formation) for that time. In most cases there will even not be the slightest trace of unspecific reaction, even after evaporation to dryness of the reaction mixture. This is, however, dependent on the use of a suitable supporting material and is only absolutely sure when the surface of the supporting sheet fulfils the conditions mentioned above, for instance in the case of using a supporting sheet covered by a thin layer of regenerated cellulose.

It may be desirable for specific purposes to include areas of other sera or to omit one or more of the areas shown. It is preferable, however, to maintain in all cases the area carrying indifferent serum, since this will unveil panagglutination and will also show that the reaction has been carried out in proper manner and that the test card has not been subjected to influences that have destroyed the capability of omitting unspecific reactions.

The card is used in the same manner as the known card by Wagner.

As an example it may be mentioned that each area may contain 60–65 mm.^{3} of the diluted salt-containing serum calculated as stated above. This serum should preferably not be spread over the whole area marked but left as a drop in the middle of the area. It should be dried at a low temperature, not exceeding 40° C. The moistening fluid should be water which may be ordinary tap water or rain water or distilled water or a saline solution. The amount thereof will preferably be for instance 50 mm.^{3} to each area. The blood should preferably be fresh blood from any part of the body, for instance from the ear lobe, and the amount thereof should preferably be 10 mm.^{3}. These amounts may be varied but they are suitable for areas of 150–500 mm.^{2}.

I claim as my invention:

1. Means for blood grouping comprising a solid sheet having at least one surface area carrying the dried residue of a mixture of a dextrane solution and a serum containing a specific agglutinin against human blood cells, the proportion of dried serum residue to that of dried solution residue being one such as would result from drying a mixture of the serum and a six percent dextrane solution having a composition between about one percent serum and about twenty percent serum.

2. Means for blood grouping comprising a solid sheet having at least one surface area carrying the dried residue of a mixture of a dextrane solution and a serum containing a specific agglutinin against human blood cells, the proportion of dried serum residue to that of dried solution residue being one such as would result from drying a mixture of the serum and a six percent dextrane solution having the composition of about one part serum and about nine parts of the six percent dextrane solution.

3. Means for blood grouping comprising a solid sheet having at least one surface area carrying the dried residue of a solution of albumin and a serum containing a specific agglutinin against human blood cells, the proportion of albumin to the serum being one such as would result from drying a mixture of the serum and a twenty percent albumin solution having a composition between about one percent and about twenty percent serum.

4. Means for blood grouping comprising a solid sheet having at least one surface area carrying the dried residue of a solution of albumin and a serum containing a specific agglutinin against human blood cells, the proportion of albumin to the serum being one such as would result from drying a mixture of the serum and a twenty percent albumin solution having the composition of about one part serum and about nine parts of the twenty percent albumin solution.

5. Means for blood grouping comprising a solid sheet having at least one surface area carrying the dried residue of a mixture comprising a low proportion of a strong test serum containing a specific agglutinin against human blood cells and a major portion made up of two different conglutinin-substitute solutions, the ratio of the dried residues of the respective solutions being one such as would result from drying a mixture consisting of about one to about twenty percent of one solution and about ninety-nine to about eighty percent of the other.

6. Means for blood grouping, comprising a solid sheet having at least one surface area carrying the dried residue of a conglutinin-substitute solution and a serum containing a specific agglutinin against human blood cells, the proportion of the dried residue of the conglutinin-substi-

tute to the dried residue of the serum being one such as would result from drying a mixture of serum and conglutinin-substitute solution containing between about one percent and about twenty percent serum.

7. Blood-grouping means as set forth in claim 5 with the addition that said solid sheet comprises a cellophane facing providing the surface area carrying said dried residue.

8. Blood-grouping means as set forth in claim 6 with the addition that said solid sheet comprises a cellophane facing providing the surface area carrying said dried residue.

9. Means for blood-grouping as set forth in claim 6 with the addition that said sheet has a plurality of such residue-carrying areas, the respective residues containing different specific agglutinins against human blood cells, and the ratio of dried serum residue to dried conglutinin-substitute residue being substantially the same in the several areas.

10. Means for blood-grouping as set forth in claim 6 wherein, in addition to said surface area carrying a residue of a serum containing a specific agglutinin against human

blood cells, said sheet also has a second surface area carrying the dried residue of a conglutinin-substitute solution and a serum free from specific agglutinins against human blood cells, the ratio of dried serum residue to dried conglutinin-substitute ratio being substantially the same in said two surface areas.

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