A STUDY OF THE PLASTIC CARD METHOD FOR THE DETERMINATION OF THE HUMAN BLOOD GROUPS

By

Erma Marguerite Hill

A THESIS

Submitted to the School of Graduate Studies of Michigan State College of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

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AN ABSTRACT

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A study of the plastic card method of Lovinson and Schultz for the determination of ABO and Rh_o blood groups was conducted to determine whether or not the technique could be safely used in a mass blood typing program. The method offered the advantage of a rapid test performed at the site of blood procurement and a permanent record of the results obtained. To evaluate the accuracy and adequacy of the plastic card method a statistically significant number of results were compared with those found by standard test tube procedures using bloods from the same individuals. The cards were of cellulose acetate, 8.5 cm. x 6.0 cm. x 0.015 mm. They were provided with spaces for the permanent record of name of the donor, date, results of the tests, and properly labelled printed circles for each of the blood grouping tests.

A preliminary study of the plastic card method for the determination of ABO and Rh_o blood groups of 8,891 individuals was performed under field conditions using locally recruited and trained field personnel. An analysis of the data indicated the potentialities of the plastic card method and sources of error in the method. The need for well trained and experienced personnel was indicated.

A second study was conducted in the field under re-defined conditions. The blood groups of 9,653 individuals were determined by the plastic card method, and the results were compared with those independently obtained by laboratory tube methods. When discrepancies existed between the results,

the laboratory blood specimens were subjected to more extensive study of the blood groups, using multiple antiserums and anti-globulin tests, and second blood specimens were obtained from the donors whenever possible.

In the final study a technical error of 0.16 percent and a total error of 0.49 percent, when inconclusive results and clerical errors were included, was found in the plastic card method for the determination of ABO and Rh blood groups as compared with standard tube methods.

The effect of red cell, antiserum, and albumin diluent concentration and the physical variables of time, temperature, and admixture was studied in a laboratory investigation of the limitations of the plastic card method.

The plastic card was examined as a medium for Rh antibody determination, Coombs anti-globulin testing, and study of weakly reacting $Rh_o(D^u)$ antigens. When the results were compared with results obtained both by standard tube and warm slide methods, it was found that the plastic card method was somewhat less sensitive than the tube methods but approximately comparable to the warm slide method both for antibody titration and for the anti-globulin test. However, the plastic card was unsatisfactory for the detection of weakly reacting $Rh_o(D^u)$ antigens.

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A STUDY OF THE PLASTIC CARD METHOD FOR THE DETERMINATION OF HUMAN BLOOD GROUPS

As a result of the advances in knowledge of red cell antigens and antibodies in human blood, blood transfusion has assumed great importance in the field of therapeutic medicine. Today there are constantly increasing demands for transfusion therapy both for the civilian population and for the armed forces. In addition, it has been anticipated in the current preparation for civil defense that administration of blood would be the chief therapeutic measure to be employed in the event of atomic bombing.

While the last decade has seen the development of highly organized blood procurement systems and transfusion facilities in which little improvement is to be expected, the detection of blood groups discovered during this period has required the constant revision of blood typing and cross matching methods and a need for their evaluation exists. The Rh factor was discovered in 1940 by Landsteiner and Wiener (1), and this antigen was soon recognized as a factor in blood transfusion reactions. Continued investigation resulted not only in subdivision of the Rh blood group but also in the identification of other new antigen-antibody systems which have been implicated as the cause of certain transfusion reactions. The Rh blood groups and the other new blood groups were relatively difficult to detect. Furthermore, when it was found that certain Rh antigens, such as D^u variants, were weak and that Rh antibodies usually could not be demonstrated in saline diluent, it became necessary to devise new techniques

for their detection. With the fundamental changes in blood typing methods has come a constant need for their evaluation in reference to particular conditions.

The current preparation for civil defense has created an individual problem in blood typing. The properly identified blood which must be ready for immediate use in any bombed area cannot be procured and stockpiled in advance. Each civilian is both potential donor and recipient, a situation which necessitates the blood typing of large populations as a civil defense measure. It has been estimated by the Civil Defense Commission of Michigan (2) that it will require the typing for ABO and Rh_o blood groups of 3,000,000 people in this state alone to provide adequate transfusion protection. The economic and temporal aspects of the blood typing involved have made it expedient to determine if blood typing tests performed in the field immediate to the individual being typed are as accurate and generally satisfactory as the laboratory methods in use. Since the safety of blood transfusion is absolutely dependent on proper blood typing and cross matching, the reliability of a testing method is of paramount importance. The danger from transfusion of incompatible ABO blood groups is immediate and fatalities occur. In an emergency such as would arise in the case of atomic disaster it might be necessary to administer blood without retyping either donor or recipient or resorting to the cross matching of bloods which is the safeguard of every transfusion. Furthermore, with potential transfusion of the general population greater emphasis must be placed on the correctness of Rh typing than when all the recipients are to be men. Although danger from immunization by incompatible Rh red cells

exists for each sex, it is recognized that the greatest harm comes from the transfusion of females before, or during, the child-bearing age with its attendent possibility of hemolytic disease for the next generation. Many babies have died of hemolytic disease as a result of prior transfusion of the mother with incompatible Rh red cells.

The slide tests which have been developed for antigen-antibody reactions are applicable to field use and are capable of a high degree of accuracy when correctly used. However, the slide test for ABO blood grouping fell into considerable disrepute for mass blood typing as a result of its use for the armed forces in World War II. Comparison of the blood grouping records of men typed at that time and the results of subsequent careful studies of their blood groups have shown discrepancies estimated to be as large as 10 percent. It is recognized that such errors probably arose not only from performance and interpretation of the test itself but also from clerical errors involved in recording the results. There are modifications of the slide test, carried out on paper, which provide permanent records and permit re-examination of both the interpretation and records of the results. ABO blood grouping has been found to be satisfactory on various forms of paper, but no adequate survey of its reliability for Rh blood grouping exists. A plastic card technique has been advocated by Levinson and Schlutz (3) for ABO and Rh blood grouping in the field. These workers found it accurate and satisfactory in their own hands and in a limited field study which they initiated. The typing of small groups of individuals in the field was performed by persons skilled in the technique, and the results were compared with those obtained in the laboratory by well

controlled methods, particularly slide methods. Before the method could be considered for use in the civil defense program, an adequate study of its reliability in field tests was necessary.

This field study was undertaken at the request of the Michigan Office of Civil Defense. The ABO and Rh blood groups of a statistically significant number of people were determined using the plastic card method in actual field conditions. The results were compared with those obtained in the laboratory by the most accurate methods available, and the accuracy of the test was evaluated. An attempt was also made to examine possible sources of error in the performance of the test in the field. In order to determine limitations of the method, a brief laboratory investigation was made of the effect of certain physical conditions and variables which might alter the results obtained by the plastic card method in the field.

It did not appear that the plastic card had been employed for the more refined Rh antigen-antibody tests. Although not the primary objective of this study, it seemed that antibody titrations, Coombs anti-globulin tests, and study of the D^u antigens on the plastic card might more sharply define the limitations and possibilities of the medium. Therefore, the potentialities of the plastic card medium for tests of this type were investigated in the laboratory.

HISTORICAL REVIEW

It was the discovery in 1900 by Landsteiner (4) of the ABO blood group antigens in man that gave rise to the concept of the individuality of human blood and provided a basis for the practice of blood transfusion. He found that the red cells of certain individuals were agglutinated by the blood serum of other individuals. Thirty years later he received the Nobel Prize for this discovery. However, it was the finding of the Rh factor, or antigen, in human blood in 1940 by Landsteiner and Wiener (1) and subsequent developments that clarified many of the hitherto unexplained transfusion reactions. These workers found that rabbits and guinea pigs immunized with the red cells of the monkey Macacus rhesus developed anti-rhesus antibodies which agglutinated the red cells of 85 percent of the white people of New York as well as the red cells of the monkey. The blood groups were designated as Rh positive or negative. Wiener and Peters (5) established the importance of the Rh antigen in blood transfusion when they demonstrated that Rh antibody was present in the serum of certain people who suffered hemolytic reactions following transfusion even though blood of the correct ABO group had been administered.

Levine and Stetson (6) had reported a case in 1939 in which a mother had been immunized against a fetal antigen that was not of the ABO, MN, or P blood groups then known, and they called the antigen of the infant a "new" antigen which the mother lacked. The antibody of the mother was later shown to be anti-Rh, and thus the fundamental role of the Rh antigen in

erythroblastosis fetalis or hemolytic disease of the newborn was established.

Continued investigation resulted in the identification of several antigens of the Rh system, designated by the American workers as Rh_o, rh', and rh'', and the corresponding Hr antigens. As early as 1941 Wiener (7), Landsteiner and Wiener (8), and Levine (9) recognized anti-Rh_o and anti-rh' serums and Levine mentioned the anti-Hr serum. These workers realized that antigenic individuality must be reflected. In 1943 Wiener and Sonn (10) identified the additional antigen rh''. The Rh_o antigen of Wiener was designated as D, the rh' as C, and the rh'' as E by the English workers.

At the end of 1943 Fisher (11) in England showed that there were six Rh antigens which he theorized fell in three pairs which he called Cc, Dd, and Ee. The theory has been confirmed by the finding of antiserum to each of the antigens. Three genes, namely one of each pair, are carried on one chromosome. The relationship between the members of each pair is one of genetic allelemorphism, that is, one chromosome carries D or d but not both. It follows, since all the nucleated cells in the body except sex cells carry a double set of chromosomes, that an individual may be either homozygous or heterozygous for each of the three Rh antigens. These six elementary antigens and C^W can be definitively determined by typing with separate specific antiserums. A third C antigen, C^W, allelomorphic to C and c, was described by Callender and Race (12) in 1946 and its position as such was clearly established by finding examples of specific anti-C^W serum.

A group of other antigens allelomorphic to C and D exist for which specific antiserums have not been found. In 1946 Stratton (13) found D antigens that were not detectable with the usual antiserums and methods of testing. These he designated as D^u antigens. They are undoubtedly the same as were described by Wiener (14) in 1944 as Rh "intermediates". While their inheritance is definitely allelomorphic, specific antiserums have not been found. There is no doubt that different grades of D^u antigens exist; some can be detected by one anti-D serum and not by others (15,16), and some can be distinguished only by use of the anti-human globulin test. In the anti-globulin test, described by Coombs, Mourant, and Race (17,18), red cells which have been sensitized by anti-D serum, but not agglutinated by it, are exposed to anti-human globulin with resultant agglutination of D positive red cells. The validity of this procedure has not been questioned for the detection of D^u variants; Rosenfield, Vogel, Miller, and Haber (19) were able to elute incomplete Rh antibody from the red cells by a modification of the Landsteiner and Miller method (20) and thus show that absorption of the antibody on the cells had occurred.

The D^u antigens are of clinical and serological importance, not merely academic, since they are D positive and have been shown by van Loghem (21) and others (19,22) to be definitely antigenic to D negative persons. They must therefore be excluded when selecting Rh negative blood donors. Few studies of the incidence of D^u bloods are available. The incidence of weakly reacting D positive (D^u) blood was found by Rosenfield et al. (19) to be approximately 1.6 percent of the white population in New York City and about 0.4 percent of these failed to react directly with any anti-D typing serum.

The antibodies, or agglutinins, normally present in human serum that are specific against the A and B red cell antigens were shown by Landsteiner to be capable of causing agglutination in saline diluent. Likewise, the antibodies produced against the M, N, and P red cell antigens, the only other blood groups known before the discovery of the Rh factor, were active in a saline medium. For four years after the discovery of the Rh antigens, the recognition of the Rh antibody was confined to that which was active in saline. In contrast to the other blood groups, the Rh antibody was more reactive at 37 C. than at lower temperatures. While it was realized that antibody was not being demonstrated in most Rh negative mothers of erythroblastotic infants who were certainly affected by the presence of anti-Rh antibodies in their circulation, it was not until 1944 that Diamond (23) reported that the concentrated globulin of an anti-Rh serum inhibited the effect of the recognized saline anti-Rh agglutinin. In the same year Race (24) and Wiener (25) independently observed the same phenomenon. Race termed it an incomplete antibody, and Wiener called it blocking antibody.

Many studies were initiated by these findings. In the meantime, although blocking or inhibition of the saline Rh agglutinins showed the presence of a different type of antibody, saline continued to be used as a diluent. Diamond and Abelson (26) demonstrated that incomplete anti-Rh serum agglutinated red cells on a slide if the cell suspensions were very heavy; the test was most successful when the red cells were suspended in their own serum or in albumin. Wiener (27) and Wiener, Hurst, and Sonn-Gordon (28) used plasma as a diluent in the "conglutination" test. In 1945

Diamond and Denton (29) studied various media, particularly those of protein nature, in which to demonstrate the incomplete antibody, and they selected 20 percent bovine albumin as the most useful. Anti-human glubulin was found (18) to be of great importance for the demonstration of certain types of Rh antibodies which do not agglutinate and do not block in saline but are usually demonstrable in albumin. Antibodies of this type have been called cryptagglutinoids by Hill, Haberman, and Guy (30) and described as "blocking in albumin" by Witebsky and Mohn (31). The latter authors (32) described a fourth type of Rh antibody which can only be identified by anti-human globulin but which does not block the reaction of the other types of Rh antibodies in saline or albumin. On these findings hinge the fundamental changes that have occurred in the methods of determination of the Rh antigen-antibody system, that is, use of Rh typing antiserums of the incomplete variety, protein diluents, and especially anti-human globulin. As a result, the methods of detection which were applicable to the Rh systems were instrumental in the discovery of other blood group systems.

Although many studies of the incidence of ABO and Rh blood groups have been made since the discovery of these red cell antigens, few direct comparisons have been published of results obtained by more than one method using blood from the same individual. Chown, Peterson, Lewis and Hall (33) compared the incidence of Rh groups which they found by the capillary method using bloods from 792 persons with the findings of Race, Mourant, Lawler, and Sanger (34) who used the tube method to determine the Rh groups of 2,000 persons. Discombe and Meyer (35) tested 1,059 bloods by the method of Chown for a similar comparison. In a preliminary study

of large scale blood typing for civil defense Allen, Diamond, and Madden (36) determined the ABO and Rh blood groups of 1,029 individuals using both a warm slide technique and test tube methods; only 215 of the total number were tested independently. They found an error of 0.08 percent by the slide method as compared with an error of 0.41 percent by the tube method.

GENERAL PROCEDURES

Test Tube Methods

The methods of the Michigan Office of Civil Defense (2) for the determination of ABO and Rh blood groups were used throughout this study. Methods similar to these have been described as "modified" tube tests in order to distinguish them from tube tests in which saline diluent is used. Antiserums of the incomplete, or hyperimmune, variety are employed in modified tube methods. These serums have been designated as "slide test" serums since anti-Rh serums containing saline-active agglutinins had previously been designated as "tube test" serums.

The antiserums used were commercially available serums of the incomplete or "slide test" variety which conformed to the potency standards of the National Institutes of Health (37,38). Each lot of all the antiserums used was tested in the laboratory for specificity and suitability for the methods with bloods of predetermined blood groups. Antiserums of the same manufacture and lot numbers were used for the tube procedures in the laboratory as were used for the corresponding plastic card tests in the field.

Determination of ABO antigens (direct typing). One drop each, 0.05 ml., of anti-A and anti-B serum was placed in 3 x 3/8 inch test tubes. To each was added an equal volume of a 2 percent suspension of the red cells to be tested in their own serum. The mixtures were thoroughly shaken

allowed to stand at room temperature for 60 minutes, and examined grossly for agglutination. The results were recorded by + or - signs.

Determination of ABO agglutinins (reverse typing). Two drops, 0.1 ml., of serum from the blood to be tested were placed in each of two 3 x 3/8 inch test tubes and heated for 15 minutes at 56 C. One drop, 0.05 ml., of a 2 percent suspension of known group A, Rh negative red cells in 0.9 percent sodium chloride solution was added to one tube, and an equal volume of a known group B, Rh negative red cell suspension was added to the second tube. The mixtures were shaken thoroughly and centrifuged for 2 minutes at 1,000 rpm. Each of the tubes was examined for agglutination, and the results were recorded.

The results of the direct ABO typing and the confirmation test were recorded without reference of one to the other. The results of the independent tests were correlated and summarized as the blood group A, B, AB, or O.

Determination of Rh_0 antigens. One drop, 0.05 ml., of anti-Rh_0 (anti-D) serum was placed in a 3 x 3/8 inch test tube. An equal volume of the 2 percent suspension of red cells in their own serum was added. The red cell-antiserum mixture was thoroughly shaken, incubated at 37 C. in a water bath for 60 minutes, and centrifuged for 1-2 minutes at 1,000 rpm. Each tube was examined grossly for agglutination, and the result was recorded by a + sign or the abbreviation Neg.

Determination of rh' (C) antigens. All specimens which were found to be Rh_0(D) negative in the aforesaid test were retested in the same manner

both with a second anti-Rh_o (anti-D) serum of different manufacture and with an anti-Rh_o'(CD) serum. If the second anti-D serum gave a positive result when the first test was negative, it was necessary to justify the existence of contrary results. Since opposite results may be a reflection of inherent differences in the strength of the antiserums or may be due to the presence of a weakly reacting Rh positive (D^u) antigen, the reactions with additional antiserums were studied, and the anti-human globulin test was applied to apparently negative bloods.

If the results with anti-CD serum were positive and if the Coombs anti-human globulin test with anti-D serum proved the cells to be D negative, the results were recorded as rh'(C) positive.

Determination of weakly reacting Rh(D^u) antigens. Equal volumes, 0.1 ml., of a 2 percent suspension of red cells in 0.9 percent sodium chloride solution and of incomplete anti-Rh_o(anti-D) serum were placed in a test tube, mixed, and incubated for 60 minutes at 37 C. Control tests were prepared in the same manner using 20 percent diagnostic albumin instead of anti-Rh_o(anti-D) serum. The cells were washed three times with approximately 5 ml. of physiological saline. Two drops of anti-human globulin serum were added to the 2 percent suspension of washed cells and mixed. After incubation at room temperature for 15 minutes, they were centrifuged at 1,000 rpm. for 1 minute and examined for agglutination.

Plastic Card Method

The plastic card used was recommended by Dr. Sidney Levinson and his co-workers. The cards were of cellulose acetate, 8.5 x 6 cm. and 0.015 mm.

thick, with properly labelled printed circles for each of the blood grouping tests.

Commercially available anti-A, anti-B, and anti-Rh_0(anti-D) "slide test" serums were used which conformed to the potency and specificity standards of the National Institutes of Health and which had been retested in the laboratory for this procedure. One drop of each serum was placed at one edge of its designated circle. One small drop of blood each for the tests with anti-A and anti-B serums and two full drops for the Rh test were placed within the circle near the antiserum. The blood and antiserums were immediately and thoroughly mixed by means of clean flat-sided toothpicks, always mixing the blood and anti-Rh serum first. The card was allowed to remain flat on the table for 2 minutes with no intervening stirring or motion. At the end of this time the card was lifted to a vertical position, the mixtures allowed to drain to the bottom of their respective circles, and the excess material was absorbed by wiping around the base of the circle with a cotton-tipped applicator. The results of the tests were read and recorded at once on the card, the International blood group as A, B, AB, or O and Rh group as Positive or Negative.

EXPERIMENTAL STUDIES

Adequacy of the Plastic Card Method Compared with Standard Tube Methods for the Determination of ABO and Rh_o Blood Groups

This field study was undertaken at the request and under the auspices of the Michigan Office of Civil Defense which was currently engaged in a blood typing program in Michigan. In order to obtain data concerning the plastic card method it was determined that parallel tests using the plastic card method and the standard Office of Civil Defense tube methods for the determination of the ABO and Rh_o(D) blood groups were to be performed on bloods from a statistically significant number of individuals. The tests by the plastic card method were to be performed at the location of the procurement of blood by a testing team which was to consist of a supervisor, one technician, and two other individuals trained in the procedure. The number of donors whose blood was to be tested by one card-typing team was not to exceed 250 per day. A blood-procurement team was to operate in conjunction with the plastic card-typing team to obtain blood specimens by venipuncture for the standard tube tests.

Organization of the Field Study

Required number of blood group determinations. This study was designed to be sufficiently comprehensive to be statistically significant in evaluating the worth of the plastic card method for establishment of the International ABO blood group and the Rh blood group. Because the two methods

of blood typing were to be performed with blood from the same donor and the one method of typing was assumed to be correct, the problem was one of checking a new procedure against an accepted correct procedure rather than a statistical evaluation of two random samples or two undetermined methods.

In considering the volume of tests required if the accuracy was to be maintained within 0.3 percent error, the problem was approached from the standpoint of group AB. Since this group has the lowest frequency, constituting only 4 percent of all ABO groups, the volume of tests required to prove accuracy for the AB group would be more than adequate for all the other groups. In order to obtain a measurable error of group AB (one incorrectly determined AB blood) it would be necessary to have approximately 10,000 tests. From this number there should be approximately 400 cases of group AB, either Rh positive or negative; a 0.3 percent error among these would allow 1.2 incorrect AB tests. Thus, with 10,000 tests it would be possible to obtain a measureable error of group AB.

However, on this same basis with 10,000 tests and a frequency distribution of 41 percent group A, 10 percent group B, and 45 percent group O, 12.3 incorrect group A, 3.0 incorrect group B, and 13.5 incorrect group O tests would be within the limits of 0.3 percent degree of accuracy.

Source of blood specimens. The initial study was conducted in the Three Rivers area and the final study, with which this report primarily deals, was conducted in the Traverse City area since each was a community in which approximately 10,000 persons could be expected to report for blood typing.

The location and hours for each blood typing clinic were scheduled in advance, by the representative of the Office of Civil Defense, to attract as many of the population as possible. Schools, factories, state institutions, and public locations for those individuals who were not members of special groups were selected as sites for bleeding clinics. Thus it was possible to obtain a test population that was representative of all age groups and which included infants, preschool children, elementary and high school children, and adults.

Organization of Donor Clinics. Blood typing clinics were so arranged that a first clerk prepared the information blank, required by the Office of Civil Defense, on which was recorded the name, address, and signature of the donor. The information blanks were assigned consecutive, identifying accession numbers. The donor carried the blank to a second clerk who recorded the identical number and name on a plastic blood-typing card. The plastic card and information blank were handed to a hostess who escorted the donor to the table where the blood types were determined by the plastic card method after the donor was properly identified with the information on the blank and on the plsstic card.

The donor was then directed to the venous bleeding station where the name on the information blank and the donor were again identified. The consecutive, identifying numbers of the information blanks had been paralleled on adhesive tape and firmly affixed to the sterile 3 inch Becton-Dickinson vacutainers which were used for the collection of venous blood specimens. The vacutainers had been placed in numerical order in

50-place metal racks and were withdrawn from the racks only in numerical order to accompany the corresponding information blank carried by the donor. After the venous blood specimen was drawn into the identified and correspondingly numbered vacutainer, it was returned to the metal rack in numerical order. The original metal rack and its numbered vacutainers of blood were sent directly to the laboratory.

Organization of plastic card blood typing. After identification of the name of the individual presenting himself for typing with that on the plastic card, one member of the typing team placed the antiserums in their respectively labelled circular areas on the plastic card. A second member of the team prepared the donor's finger by sponging it with 70 percent ethanol and punctured the finger tip with a sterile Bard-Parker blade. Without necessarily waiting for a free-falling drop, drops of blood were expressed and touched to the circular areas on the card without touching the antiserum. After the performance of the test as described under General Procedures and the recording of the results on the card, the card was placed vertically in a groved board which held 25 cards. Each card was placed in numerical order. When the serum-blood mixture was completely dry, each series of 25 cards was reviewed, packaged together with an outer label bearing the series numbers, date of examination, and the initials of the supervising bacteriologist. All the card typings were sent each day to the laboratory where they were re-examined for clerical and technical errors before comparisons were made with the results of corresponding tube tests which were performed and

recorded independently in the local testing laboratory in the Three Rivers study and in the Laboratory of the Michigan Department of Health, Lansing, in the Traverse City study.

Results

The initial study of the plastic card method for blood typing in the field was undertaken to determine whether or not the plastic card method could be employed in a large scale typing program using locally recruited and trained personnel of average ability and training. The initial instruction in the use of the method was demonstrated by Dr. Sidney Levinson. The supervisory personnel of the card-typing teams were persons with a college background and some training and experience in laboratory procedures, including that of blood typing in small hospitals. A group of 6,691 individuals was tested in the Three Rivers area during a 6-week period. The parallel blood typing by the standard Office of Civil Defense methods was performed in a local laboratory which was established as were the other laboratories for the Michigan Office of Civil Defense blood typing program. Two venous blood specimens were obtained from 10 percent of the first hundred donors and from 2 percent of all subsequent ones; the duplicates were submitted to the Michigan Department of Health Laboratories for checking of accuracy.

A summary of the results obtained in the study conducted in the Three Rivers area is presented in Table 1. The results of 6,455 blood typings performed by the plastic card method were in agreement with the results obtained by the standard tube methods. It may be noted in the

TABLE 1

A PRELIMINARY STUDY OF 7191 BLOOD GROUP DETERMINATIONS BY THE PLASTIC CARD METHOD AS PERFORMED UNDER AVERAGE SUPERVISION

	Number	Percent
Individuals tested	7,191	
Technically satisfactory tests	6,803	95.5
Technically correct results	6,456	95.0
Technically unsatisfactory and inconclusive tests	387	4.4
Errors in clerical transcription	30	0.32
Errors in ABO grouping tests	1	0.01
Errors in Rh$_0$ grouping tests	17	0.2

table that 388 typings by the plastic card method were discarded. Since these were classified with the errors, it constituted an immediate error of 4.4 percent. Three hundred and twenty-five of these results were discarded because incorrect technique was used on two successive days when a technician experienced only in the slide method of blood typing was allowed to substitute on one team. The technician adamantly followed a technique used in slide methods for blood typing; the blood and antiserums were mixed by a rotary motion and allowed to dry on the card instead of draining off the excess material. The results were somewhat obscured. In most instances the results could be read and were recorded. A clerical error of 0.34 percent was found; with these, the results were evident and satisfactory but incorrectly recorded on the card. The error due to an actual discrepancy between the results found by the two methods was 0.21 percent.

When the results found by the plastic card method were analyzed and the total performance of the test in the field was examined, it was evident both that the plastic card method possessed the potentialities of a desirable method and that there were inherent dangers in the method unless the blood typing was conducted by adequately trained and experienced personnel. It was also recognized that parallel tube testing of the highest degree of accuracy would be more readily obtained in a permanent laboratory prepared to perform the more refined Rh antigen-antibody studies.

A study of this nature was undertaken in the Traverse City area where the blood specimens of 9,653 individuals were tested both by the plastic card method and by the standard Office of Civil Defense

procedures. Well qualified bacteriologists had been carefully trained in blood typing procedures and in the plastic card technique by the author. The tests performed on the plastic cards were examined and the results were recorded at the place of blood procurement by testing teams under the direct supervision of the responsible bacteriologists. The standard Office of Civil Defense blood typing was performed in the Laboratories of the Michigan Department of Health under the direct supervision of the author. In addition, all the plastic card test results were re-examined by the author, upon submission of the cards each day, for verification of the interpretation of the results, and the results were then compared with the tube test results which had been independently tested and recorded.

A summary of the results obtained in the Traverse City area is presented in Table 2. The results of 7,010 of the blood typings performed by the plastic card method were in agreement with the results obtained by the standard tube method. The recorded results of 15 blood typings performed by the plastic card method did not agree with those obtained by the tube method. The results of 24 of the A1 tests on the cards were inconclusive; therefore, no results were recorded in the field for these bloods. Three clerical errors were observed on the cards; these were incorrect records of the evident results.

In every case where discrepant or inconclusive results existed, the blood typings were immediately repeated from the venous specimens in the laboratory. At least three different anti-B serums and anti-CD serums were used for these repeat tests. The bloods were also tested with

TABLE 2

ACCURACY OF 9,053 BLOOD GROUP DETERMINATIONS BY THE PLASTIC CARD METHOD

	Number	Percent
Individuals tested	9,053	
Results in complete agreement	8,810	97.34
Results inconclusive	28	0.29
Results discrepant	15	0.16
Error in ABO grouping	1	0.01
Error in Rh$_0$ grouping	14	0.15
Results with clerical error	3	0.03

anti-C and anti-E serums since the weak D^u antigen is frequently associated with C and E antigens. If indicated, the anti-human globulin test was performed using several different incomplete Rh antiserums.

Of the 2[illegible] inconclusive results in which the plastic card tests were not considered definitely negative or definitely positive, 10 bloods were $Rh_o(D)$ negative in the tube test and also negative when tested with the Coombs anti-human globulin test. Eighteen of the bloods were proved to be Rh_o positive by the tube test. However, three of these were D^u variants.

In the discrepant results, which are summarized in Table 2, there was one blood grouping which was recorded as group AB on the card but was found to be group B by the test tube procedure. This single ABO blood grouping discrepancy represented an error in judgment in the field. The plastic card test was unsatisfactory in appearance, and it should have been repeated immediately in the field.

The other 14 discrepancies between the results obtained in the field by the plastic card method and in the laboratory by the test tube method were discrepancies in Rh blood types. Five of the results on the plastic card were read as $Rh_o(D)$ positive. The test tube procedures showed that two of these bloods were $Rh_o(D)$ and rh'(C) negative and three of them were $Rh_o(D)$ negative, rh'(C) positive. Nine of the 14 bloods with which discrepancies occurred in Rh typing were recorded as Rh negative on the plastic card, but they proved to be Rh positive when tested by laboratory tube procedures. However, six of these nine bloods were "low grade" D^u variants which were detected in the laboratory only

by the use of more than one anti-D and anti-CD serum. They were definitely established as D^u variants by the Coombs anti-globulin test.

It had been observed in the Three Rivers study that many agglutinations on the plastic card were not optimum although the correct results could usually be arrived at by careful observation. In establishing standards for the plastic card testing which was to be performed at Traverse City, it was determined that every test must appear satisfactory, the criteria being either adequate, well-defined agglutination or definite lack of agglutination. Otherwise the test was to be repeated before releasing the donor. If the test was still unsatisfactory, no result was to be recorded. In an endeavor to meet this standard, the plastic card testing teams repeated 147 Rh tests and 6 ABO tests. As may be observed in Table 3 only 16 examinations, all of which were Rh tests, were inconclusive after the second test. There were 10 additional Rh tests which were considered inconclusive on the first test and should have been repeated. The number of tests repeated in the field corresponds to 22 specimens from the same series of bloods which wer re-examined in the laboratory in order to verify results or to attain completely satisfactory tests.

It would have been desirable to repeat the blood typing of all the individuals in which inconclusive or discrepant results were obtained. However, a second card typing and a second venous blood specimen for laboratory testing could only be obtained from 10 of the 15 individuals whose blood tests had resulted in discrepant results by the two methods. It may be noted in Tables 4 and 5 that when these bloods were typed on

TABLE 3

ADEQUACY OF THE PLASTIC CARD METHOD FOR THE DETERMINATION OF ABO AND Rh_0 BLOOD GROUPS

	Number	Percent
Individuals tested	[illegible]53	
Original card tests apparently satisfactory	[illegible]490	9[illegible].31
Card tests repeated at time of original test	193	1.81
Rh_0 grouping	147	1.52
ABO grouping	6	0.06
Card tests inconclusive after repeated test	18	0.19
Card tests inconclusive; repeat not obtained	10	0.10

TABLE 4

ANALYSIS OF Rh BLOOD GROUP RESULTS ORIGINALLY DISCREPANT IN PLASTIC CARD AND LABORATORY METHODS OF DETERMINATION

Specimen Number	Original examination: Result Recorded on Plastic Card	Original examination: Re-analysis of Plastic Card Result	Original examination: Result Reported by Laboratory		Repeat Examination: Plastic Card	Repeat Examination: Laboratory	
	Rh_0(D)	Rh_0(D)	Rh_0(D)	rh'(C)	Rh_0(D)	Rh_0(D)	rh'(C)
187	+ ?	Neg.	Neg.	Neg.	Not repeated		
2,497	+	Unsat.	Neg.	Neg.	Neg.	Neg.	Neg.
2,942	+ ?	Unsat.	Neg.	+	Neg.	Neg.	+
3,059	+	+	Neg.	+	Neg.	Neg.	+
3,272	+	Unsat.	Neg.	+	Neg.	Neg.	+
1,234	Neg. ??	Unsat.	+		Not repeated		
1,500	Neg. ??	+	+		Not repeated		
2,749	Neg. ?	Unsat.	+		Not repeated		
197	Neg.	Unsat.	+(D^u)		Neg.	+(D^u)	
3,430	Neg.	Unsat.	+(D^u)		Neg.	+(D^u)	
4,043	Neg.	Neg.	+(D^u)		Neg.	+(D^u)	
2,060	Neg.	Unsat.	+		+	+(Wk)	
2,555	Neg.	Neg.	+(D^u)		Neg.	+(D^u)	
2,917	Neg.	Neg.	+		Not repeated		

TABLE 5

ANALYSIS OF ABO BLOOD GROUP RESULTS DISCREPANT BY PLASTIC CARD AND LABORATORY METHODS OF DETERMINATION

Specimen Number	Original Examination			Repeat Examination	
	Result Recorded on Plastic Card	Re-analysis of Plastic Card Result	Result Reported by Laboratory	Plastic Card	Laboratory
1102	AB	Unsat.	B	B	B

plastic cards, the results of five of the Rh tests and the one ABO test, which was technically unsatisfactory on the first examination, did not agree with the original results obtained by the plastic card method. They did agree both with the original and the duplicated results obtained on blood from the same individuals by laboratory tube procedures. These five results were classified as technical errors in the performance of the original card test. When the other 4 of the 10 bloods were retyped by the plastic card method, the same results were obtained as had been found with the original plastic card test; all bloods appeared to be Rh negative. These four results on the plastic card were in disagreement with the $Rh_0(D)$ positive results obtained by the laboratory tube procedures. However, the correct $Rh_0(D)$ positive typings were ascertained only after application of several antiserums and the use of the anti-human globulin test, Table 6. If these four results had been compared with the results from only one tube test or from one of lower sensitivity, they would likely have been found to be in agreement. Nevertheless, these four results were considered as errors inherent in the plastic card method.

A review of all the parallel results showed that of the 15 blood typing results determined by the plastic card method which did not agree with the tube method results, 11 had been considered of questionable value before comparison of the parallel results. Blood groups had been recorded on five plastic cards which the field bacteriologists annotated as tests of dubious value. An additional six results were considered unsatisfactory by the author on review of the card tests prior to comparison with the laboratory results. These 11 plastic card typings

TABLE .

RH TYPING OF 14 BLOOD SPECIMENS USING MULTIPLE TUBE TESTS

Specimen	Degree of Agglutination														Rh Groups	
	Routine Test			Supplementary Tests						Anti-Globulin Tests						
	Incomplete Serums			Incomplete Serums			Saline Agglut. Serums			Incomplete Anti-D Serums						
	Anti-D		Anti-CD	Anti-D			Anti-D	Anti-C	Anti-E							
	1*	2		3	4	5				1	2	3	4	5	Rh_0(D)	rh'(C)
187	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Neg.	Neg.
797	-	2	4	±	2	1	-	-	-	3	4	4	4	3	+**	
1,234	2	4	4	3	4	3	1	4	-	4	4	4	3	4	+	
1,560	4	4	4	4	4	4	4	4	-	4	4	4	4	4	+	
2,729	1	2	4	2	-	±	-	4	-	4	4	4	4	4	+**	
3,436	-	-	4	±	2	1	-	4	-	3	3	2	3	3	+**	
4,043	1	±	4	1	±	-	-	4	-	2	3	3	2	±	+**	
5,497	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Neg.	Neg.
6,982	-	-	4	-	±	-	-	4	-	-	-	-	-	-	Neg.	+
7,060	-	1	4	±	4	2	±	4	-	4	3	4	4	2	+**	
7,655	±	±	4	1	±	1	-	3	-	3	3	4	3	3	+**	
7,659	-	±	4	-	-	-	-	4	-	-	-	-	-	-	Neg.	+
7,672	-	-	4	-	-	±	-	4	-	-	-	-	-	-	Neg.	+
8,967	2	2	4	2	3	2	-	4	-	4	4	4	4	4	+**	

* Serum used for plastic card test.

** Weakly reacting Rh positive (D^u) bloods.

should not have been recorded as conclusive on the basis of a single sample. Among the plastic card tests of dubious value were two recorded as $Rh_o(D)$ positive, bloods 5497 and 167, which were entirely negative in laboratory tests. When blood 5,497 was retested by the plastic card method, it appeared negative. The results with blood 167 were not considered discrepent when the original test was re-examined so the test was not repeated; it appeared to the reviewer to be Rh negative on the original card, and the error was thus one of interpretation and recording of the results.

Of the four discrepent results which appeared to be satisfactory on the original plastic card, one blood (No. 7,659) was designated as $Rh_o(D)$ positive on the card but it was persistently $Rh_o(D)$ negative, rh'(C) positive on laboratory testing; repeated tests on three plastic cards were $Rh_o(D)$ negative. The three remaining discrepancies in results were from bloods that appeared to be satisfactory $Rh_o(D)$ negatives by the plastic card method and also gave negative or weak reactions with the same antiserums in the first tube procedure. The correct results, $Rh_o(D)$ positive, were only recognized because all bloods which appeared to be Rh negative were routinely subjected to additional tests in the laboratory procedure.

In this study the determination of ABO and $Rh_o(D)$ blood groups by the plastic card method exhibited a technical error of 0.16 percent and a total error of 0.49 percent, when inconclusive results and clerical errors were included, as compared with results found by the laboratory test tube methods.

Potentialities and Limitations of the Plastic Card Method

It is well known in the field of serological testing that such factors as temperature, time, diluent, ratios of reacting substances and even the method of combination of reagents frequently have a significant influence on the results. Therefore, in order to test more completely the potentialities and define the limitations of the plastic card as a vehicle for the antigen-antibody reactions of blood grouping, laboratory studies were made of certain of the variables which might influence the results of the plastic card tests. In view of the fact that agglutination in the Rh system is more difficult to analyze, emphasis was placed on examination of the Rh antigen-antibody reaction.

Effect of Red Cell Concentration

Levinson and Schlutz (3), who advocated the plastic card for blood grouping, suggested in a personal communication that two drops of fresh blood and one drop of anti-Rh_o serum be used routinely for the determination of Rh types by the plastic card method and that three drops of the blood of anemic individuals might be required for optimum agglutination. On the other hand, they recommended less than a drop of blood, a small amount on a flat toothpick, as a suitable volume to add to one drop of anti-A and anti-B serums. Allen, Diamond, and Madden (30) used a "very small" amount of blood to determine ABO groups by the slide method. It was observed in the preliminary field study at Three Rivers that when a larger volume of blood than that recommended was inadvertently added to the serum for ABO grouping, the resulting agglutination

appeared more distinct. It seemed desirable to determine the optimum amounts of antigen and antibody with as quantitative a procedure as the plastic card medium permitted.

Rh positive red cells were centrifuged until there was no change in volume of the packed cells. Suspensions of the packed red cells ranging from 15 to 60 percent were prepared in group specific serum. A commercially prepared incomplete anti-Rh_o(anti-D) serum which conformed to the National Institutes of Health requirements was used for the tests. It was necessary to pipette one amount of serum and one amount of red cell suspension at a time and to proceed with the mixing, timing, and draining of the serum-cell mixture. Because of the size of the defined area within the circles on the plastic card, a total volume of 0.2 ml. was the feasible limit of quantity that could be used. To each of three defined areas on plastic cards was added 0.05 ml. of anti-Rh_o serum and to a fourth was added 0.1 ml. of the serum. The red cell suspension of a given concentration was added to the serum using 0.05, 0.10, and 0.15 ml. with the 0.05 ml. amounts of serum and using 0.10 ml. of red cell suspension with the 0.10 ml. of serum. The red cells and serum were thoroughly mixed, and a time interval of exactly 2 minutes was allowed between the mixing and draining of the excess material from the card. The experiment was repeated several times. The effect of red cell concentration on ABO reactions on the plastic card was examined in a similar manner. Representative data are presented in Tables 7 and 8.

It was observed that a considerable latitude in the proportions of red cells and serum was possible. However, 0.05 ml. of anti-Rh serum

TABLE 7

EFFECT OF RED CELL CONCENTRATION ON Rh REACTIONS ON THE PLASTIC CARD

Volume of Red Cell Suspension, ml.	Volume of Anti-Rh, Serum, ml.	Degree of Agglutination in 2 minutes						
		Percent Red Cell Concentration in Autologous Serum						
		60	50	40	30	25	20	15
0.05	0.05	4	4	4	3	1	1	±
0.10	0.05	3*	4	4	3	3	1	±
0.15	0.05	3*	4	4	4	3	1	±
0.10	0.10	3*	4	4	4	2	1	±

* Viscous.

TABLE 8

EFFECT OF RED CELL CONCENTRATION ON ABO REACTIONS ON THE PLASTIC CARD

Volume of Red Cell Suspension, ml.	Antiserum		Degree of Agglutination in 2 minutes						
			Percent Red Cell Concentration in Autologous Serum						
	Specificity	Volume ml.	60*	50	40	30	25	20	15
0.025	Anti-A	0.05	0	4	3	3	2	2	1
	Anti-B	0.05	0	4	4	3	2	2	1
0.05	Anti-A	0.05	0	4	4	4	4	3	2
	Anti-B	0.05	0	4	4	4	4	3	2
0.10	Anti-A	0.05	0	4	4	4	4	4	3
	Anti-B	0.05	0	4	4	4	4	4	3

* No test.

and 0.10 ml. of 40-50 percent red cell suspension was the optimum ratio for the interval of 2 minutes. Red cell suspensions of more than 50 percent concentration with this amount of serum were viscous and the mixture did not drain from the cards easily to give well separated agglutination particles. With red cell suspensions of 25 and 30 percent the agglutination was satisfactory but less visible. With red cell suspensions of 15 and 20 percent the agglutination was unsatisfactory. When 0.10 ml. of anti-Rh serum and 0.10 ml. of 40-50 percent of red cell suspension were used, satisfactory results were obtained, but it unnecessarily increased the quantity of antiserum required.

Since normal whole blood contains approximately 45 percent of red cells, the ratio of one part of anti-Rh serum to two parts of 40-50 percent red cell suspension which was found to be optimum in the quantitative study corresponds to the amounts recommended by Levinson and Schlutz for routine Rh testing by the plastic card method. However, it appeared in the present studies that the recommendation of Levinson and Schlutz and of Allen et al. that a very small volume of blood be used for ABO blood group determination was not only unnecessary but even undesirable for the plastic card method for the determination of ABO blood groups.

Effect of Albumin Content of Anti-Rh Serum

The usefulness of albumin as a diluent in the detection of Rh antigens with incomplete Rh antibody was originally demonstrated by Diamond and Denton (29). The commercially available incomplete anti-Rh serums

in present use are usually diluted with albumin in their preparation. It had been observed that certain anti-Rh serums used for the plastic card method of typing were more viscous and dried more readily than others. When the albumin concentration of five samples of commercially available anti-Rh serums was determined and found to range from 21-30 percent, the question arose as to the effect this variation might have on the reactivity of the Rh testing serums used in the method under study.

To examine the effect of albumin concentration on reactivity, the titers of individual serums were to be determined after dilution of the serum with albumin of several concentrations. Commercially available anti-Rh serum could not be used because it already contained albumin. Therefore, three anti-Rh serums, each of which had been produced by immunization of Rh negative male individuals in the course of experimental production of diagnostic antiserums, were selected for study. The serums were first absorbed free of ABO antibodies using Rh negative red cells. Twofold serial dilutions were then prepared in 10, 15, 20, 25, and 30 percent albumin which had been tested and found suitable for use as a diagnostic reagent. Each serum in each concentration of albumin diluent was tested both by the plastic card method and by tube titration procedures.

While the reactivity on the plastic card was maximum when the antiserums were diluted in 30 percent albumin, the antiserum and blood dried quickly at warm temperatures. The reactions were obscured by failure of the fluid to drain readily from the card. The tube titrations were more

sensitive when diluted with 25 than with 30 percent albumin. The reactivity of a typical serum in the several concentrations of albumin diluent is recorded in Table 9. Progressively decreasing reactivity was observed with decreasing albumin content. The titration endpoint of the serum shown in Table 9 was 1:192 when diluted in 30 percent albumin as contrasted with an endpoint of 1:6 when diluted in 10 percent albumin. The same relationship existed between sensitivity and albumin content in each of the three serums studied. The commercially prepared serums which contained 25 and 27 percent albumin were considered more satisfactory in routine use than serum which contained 31 percent albumin.

Effect of Physical Factors

Time. It is recognized that agglutination of group A and group B red cells, with the possible exception of subgroup A cells, occurs almost immediately with anti-A and anti-B serums of the potency required to meet the present standards of the National Institutes of Health. To produce visible agglutination of Rh positive red cells by the antiserums now available, longer intervals of time are required. Levinson and Schultz has recommended that 2 minutes be allowed to elapse before rocking the cell-serum mixture from the plastic card.

To examine the influence of the time interval on red cell agglutination on the plastic card, 45 percent suspensions of group A, group B, and Rh positive red cells were prepared in Rh$_0$ specific serums. One part each, 0.05 ml., of anti-A, anti-B and anti-Rh$_0$ serums were placed within plastic-card circles. One part of the suspensions of group A or

Table 9

EFFECT OF ALBUMIN CONCENTRATION ON REACTIVITY OF ANTI-Le^a SERUM

Percent Albumin	Degree of Agglutination: Dilution of Serum in Albumin: 2	4	8	16	32	64	128	256	512	1,024	2,048	4,096	8,192	16,384	32,768	Control
Plastic Card																
30	4	4	4	4	4	4	4	4	3	3	2	1	1	-	-	-
25	4	4	4	4	4	4	4	4	3	3	1	±	-	-	-	-
20	4	4	4	4	4	4	3	2	2	1	-	-	-	-	-	-
15	4	4	4	4	4	3	3	2	2	±	-	-	-	-	-	-
10	4	4	4	4	4	2	1	1	±	-	-	-	-	-	-	-
Test tube																
30	4	4	4	4	4	4	4	4	4	3	2	2	2	-	-	-
25	4	4	4	4	4	4	4	4	4	4	3	2	2	2	1	-
20	4	4	4	4	4	4	4	4	4	3	2	2	1	-	-	-
15	4	4	4	4	4	4	4	4	3	2	1	1	±	-	-	-
10	4	4	4	4	4	4	4	3	2	1	1	±	-	-	-	-

group B cells and two parts of Rh positive red cells were used with the correct antiserums. The antiserum and red cell suspension was mixed together for exactly 30 seconds, and the period was timed until the mixture was drained from the card. The maximum time tested was limited by drying of the cell-serum mixture on the card, and it was dependent on temperature and humidity to a considerable extent.

The interval of time that elapsed was not an important factor with the group A and group B red cells tested. No attempt was made to study the effect with subgroup A red cells. Periods of time less than 2 minutes were not adequate for maximum, or optimum, agglutination of Rh positive red cells by anti-Rh serum. Agglutination was satisfactory with intervals of time of two minutes or longer, to the time limited by drying. Therefore, the safest time for optimum agglutination was an interval of 2-3 minutes between completion of stirring and draining the cell-serum mixture from the plastic card. This corresponded well with the interval of time suggested by Levinson and Schlutz.

Temperature. Since a controlled temperature is not available in the [illegible], the wide variations in temperature would undoubtedly be encountered in field use of the method, it seemed important to determine quantitatively the effect variations in temperature might have on the agglutination of red cells on the plastic card. It is known that Rh antibodies are more reactive at warm temperatures than at cold; therefore, their use in relatively cold locations might be an important source of error. To attain a quantitative comparison of the effect of temperature on the agglutination of red cells by antiserums, it was

necessary to determine the highest dilution of antiserum which agglutinated red cells to the same degree at given temperatures, or the effect of temperature on the titers of the antiserums.

Commercially prepared anti-Rh$_0$(anti-D) serums of the incomplete, or "slide test", variety were studied. Serial twofold dilutions of six sera were prepared in 25 percent albumin and then tested at 18, 25 and 37° C. The laboratory was maintained at the respective temperatures, and all the materials used in the procedure were allowed to attain these temperatures before they were used. The technique of testing described under General Procedures was strictly observed for each test.

The results of representative titrations at the different temperatures are presented in Table 10. As the temperature decreased from 37° to 18° C. the antiserum became less reactive. Anti-Rh$_0$(anti-D) serum #1 exhibited an agglutination titer (at least 1 + agglutination) of 1:128 at 37° C., 1:32 at 25° C., and 1:1 at 18° C. Similar decreases in reactivity as the temperature decreased were found with the other commercial anti-Rh$_0$ serums. It was concluded that antiserums for use in this test must be of sufficiently high titer to permit the maintenance of satisfactory reactivity in the range of temperature that might be encountered. The six Rh antiserums studied exhibited complete agglutination in dilutions of 1:2 and 1:4 even when the temperature was maintained at 18° C. Evidently Rh antiserums with titers that conformed to the National Institutes of Health standards were capable of adequate reactivity and would not be a source of error under normal variations of temperature.

Although the enhancing effect of warm temperatures was evident in these titrations, it was somewhat counteracted by the adverse effect of

TABLE 10

REACTIVITY OF INCOMPLETE ANTI-Rh_0 SERUMS AT DIFFERENT TEMPERATURES

Serum Number	Temperature °C.	Degree of Agglutination: Dilution of Serum in Albumin 2	4	8	16	32	64	128	256	512	Control
161	18	4	4	3	1	±	-	-	-	-	-
	25	4	4	3	2	1	±	-	-	-	-
	38*	4	4	4	3	2	1	1	-	-	-
1,167	18	4	4	1	±	-	-	-	-	-	-
	25	4	4	4	3	1	±	-	-	-	-
	38*	4	4	4	4	3	2	1	-	-	-
257	18	4	4	2	1	±	-	-	-	-	-
	25	4	4	4	3	1	±	-	-	-	-
	38*	4	4	4	4	3	2	1	±	±	±

* Test mixtures drying.

rapid drying of the cell-serum mixture at the higher temperatures. This readily leads to false interpretations of the results and constitutes a possible source of error.

Other factors. The effects of certain other physical factors were examined. It was readily observed that a thorough mixture of antiserum and blood was necessary if uniform sensitization and agglutination of the red cells was to occur. It was found that the subsequent treatment of the serum-cell mixture on the plastic card had marked effects on the results. The agglutination was not apparent unless the excess fluid was removed from the card. When the blood and antiserum were rotated, or slowly rocked, and allowed to stand on the card without removing the excess, the results were completely unsatisfactory. The observation of results obtained by the latter technique, which is used in slide methods, is dependent on the agglutination being visible through a transparent medium. Observation of the agglutination of red cells on the plastic card depends upon an adherent pattern of agglutination.

Sensitivity of the Plastic Card Method Compared with Certain Laboratory Procedures

The detection and quantitative determination of Rh antibodies in the serums of persons who have been sensitized by Rh antigens and the detection of weakly reacting Rh positive (D^u) antigens, either by single-stage typing or by the application of the Coombs anti-globulin procedure, require relatively refined techniques as compared with those necessary to determine the more readily typed Rh blood groups. While it was not

expected that the plastic card method would be suitable or desirable as a standard method for these determinations, they afforded an opportunity to study the capabilities and limitations of the plastic card method. The plastic card method had proved unexpectedly sensitive in quantitative tests involving albumin diluent concentrations, and throughout the laboratory study it had been noted that there was little difference between the results obtained by the plastic card method and by the slide method. Since the warm slide method had been proposed for the typing of large populations, particularly in civil defense preparation, by Elliott and Griffitts (39) and by Allen, Diamond, and Madden (36), it was included in the parallel comparison of test tube method, plastic card method, and warm slide method.

Rh antibody titration. Six anti-Rh_o typing serums of the incomplete variety that were prepared by several commercial manufacturers and 20 serums from pregnant women in which anti-Rh antibodies had been detected were subjected to parallel testing. Twofold serial dilutions of the serums were prepared in 1-ml. or 2-ml. volumes of 25 percent diagnostic albumin since this percentage approximated the average albumin content of representative commercial serums. A separate pipette was used for the preparation of each dilution of serum. Using washed Rh_o (D) red cells and pooled group specific serum, a 45 percent suspension of red cells was prepared for use in the plastic card and warm slide tests and a 2 percent suspension for use in the tube test. Each series of serum dilutions was tested by the tube method of the National Institutes of Health for the standardization of anti-Rh serums. For

this method 0.1 ml. of each serum dilution and 0.1 ml. of the 2 percent red cell suspension were combined, thoroughly mixed, incubated in a water bath at 37 C. for 60 minutes, and centrifuged for 1 minute at 1000 rpm. Each tube was examined over a flourescent light for gross agglutination, taking care to handle the tube and contents gently. The titer was recorded as the last serum dilution in which 1-plus agglutination remained for 15 minutes after the first reading. Each dilution of serum was also tested by the plastic card method described under General Procedures and by the warm slide method described by Griffitts, Elliott, and Cox (40).

The results of the three comparative tests are shown in Tables 11 and 12. The six commercial antiserums, which usually represent combined serums, exhibited titers of 1:64 to 1:128 when tested by the National Institutes of Health tube procedure. While little difference was evident in the titers obtained by the plastic card and warm slide methods, the titers were one or two twofold dilutions lower than those obtained with the tube procedure. The same relative sensitivity was observed in the titers found for serums of individuals. Those serums in which a titer of only 1:2 or 1:4 was found with the tube test were negative by both plastic card and warm slide methods.

The finding that antibody determinations on the plastic card were usually positive only one or two twofold dilutions less than with the standard tube test verified the relatively high sensitivity of the plastic card method which had been indicated elsewhere. That the results with the plastic card method were comparable to those with the warm slide

TABLE II

REACTIVITY OF ANTI-Rh SERUMS AS DETERMINED BY THE PLASTIC CARD, WARM SLIDE AND TUBE METHODS

Serum	Method	Degree of Agglutination										
		Dilution of Serum in Albumin										Control
		2	4	8	16	32	64	128	256	512	1,024	
Hoy	Card	4	4	3	2	1	-	-	-	-	-	-
	Slide	4	4	4	3	1	-	-	-	-	-	-
	Tube	4	4	4	3	2	1	±	-	-	-	-
Fe	Card	±	-	-	-							-
	Slide	±	-	-	-							-
	Tube	3	1	±	-							-
Ja	Card	4	4	4	4	3	3	1	-	-	-	-
	Slide	4	4	4	4	4	3	1	±	-	-	-
	Tube	4	4	4	4	4	4	3	2	1	±	-

TABLE 12

COMPARATIVE Rh ANTIBODY TITRATION VALUES OF 6 BLOOD GROUPING SERUMS AND 20 INDIVIDUAL SERUMS DETERMINED BY THE PLASTIC CARD, WARM SLIDE AND TUBE METHODS

Serum	Antibody Titer		
	Plastic Card	Warm Slide	Tube
Lel	32	32	64
Leo	32	32	64
257	32	16	64
1,148	32	32	128
1,401	16	32	64
1,602	16	32	32
Fe	-	-	4
Ja	128	128	512
Li	4,096	4,096	8,192
Ba	512	256	512
Bu	2	-	16
Lo	16	32	64
Mi	-	-	2
Ma	32	16	64
Le	32	32	128
Mi	-	2	
Se	128	256	256
Mi	128	128	256
Ei	16	16	32
Ad	256	512	512
Bo	64	32	128
Ev	2,048	2,048	4,096
Em	1,024	2,048	4,096
Lu	-	2	8
Pe	-	-	4
Wi	4	8	12

method also showed that the test was capable of a high degree of sensitivity. Although the warm slide method has not been recommended as a substitute for the test tube technique for antibody titration, it has been considered reasonably accurate, particularly for the preliminary estimation of titer, and is purported to have the advantage of eliminating zone phenomena in high-titered serums (40).

Detection of weakly reacting Rh positive (D^u) antigen. The plastic card method is designed for use of fresh capillary blood, but since it had been possible to demonstrate good reactions with bloods submitted for laboratory testing, even with diluted antiserums, an attempt was made to compare a series of bloods designated as D^u variants by the blood grouping laboratory.

The first series of 22 bloods were of the low grade D^u group. They had been found negative by two different anti-Rh_o(anti-D) serums, and they had been tested by the anti-human globulin procedure when they were found to be positive with an anti-Rh_o'(anti-CD) serum. Each blood sample consisted of free cells in its own serum. As is customary for warm slide testing in the absence of oxalated blood or fresh capillary blood, the sample was centrifuged and a portion of serum was removed to prepare a 40-50 percent suspension of the red cells. Each blood sample was tested both by the plastic card and by the warm slide methods. Neither procedure was effective in detecting any reaction with the most potent commercial anti-Rh_o serum available, as may be seen in Table 13. The same anti-Rh_o serum had yielded negative results in the one-stage tube test.

TABLE 13

Rh_o(D) TYPING OF WEAKLY REACTING Rh POSITIVE BLOODS

Number of Specimens	Routine Tube			Plastic Card			Warm Slide		
	Anti-Rh_o(D)	Anti-Rh_o'(CD)	Anti globulin	Positive	Negative	Inconclusive	Positive	Negative	Inconclusive
22	-	+	+	0	22	0	0	22	0
50	Wk +	+	+	34	9	7	36	7	7

A second series of 50 bloods was of the weakly reacting Rh_o group. One or both of the tests with two different anti-Rh_o serums had been doubtfully positive, or weakly reacting, and the bloods had been confirmed as Rh_o(D) positive by the Coombs anti-globulin procedure. They were tested by both the plastic card and the warm slide methods. Of the 50 bloods tested by the plastic card method, 34 were Rh_o(D) positive, 9 were Rh_o(D) negative, and 7 were inconclusive. When these same bloods were tested by the warm slide method, 36 were Rh_o(D) positive, 7 were Rh_o(D) negative, and 7 were inconclusive. Again the results obtained with the plastic card and warm slide methods were similar, but only 70 percent of the weakly reacting Rh_o(D) positive bloods were detected by these methods.

Coombs anti-globulin test. The coombs anti-globulin tests performed during the field study described were made with an anti-human serum reagent which was standardized only for tube testing. A variety of the reagent standardized for use either in test tube or slide tests has since become available. Use of this reagent confirmed the opinion of some workers that the detection of the antibody coated on the red cell but not producing agglutination, as is the case with the D^u variants of Rh positive bloods, was better determined on a slide or tile than in the tube.

A comparison was made in the laboratory of the warm slide and plastic card methods for the detection or confirmation of the presence of D^u blood types using this new anti-globulin reagent. Ten bloods which were D^uvariants of the "low grade" group used in the previous experiment were

tested. Duplicate preparations of 0.4 ml. of 2 percent suspension of the red cells in saline were sensitized with an equal volume of incomplete anti-Rh_o(anti-D) serum by incubation in test tubes in a water bath at 37 C. for 60 minutes. Red cell controls of each blood were included in which 20 percent albumin was used instead of the anti-Rh_o serum. The cells were washed three times with 0.9 percent sodium chloride solution. After the last centrifugation the saline was removed as completely as possible to leave a 40-50 percent suspension of red cells. Instead of placing the drop of reagent serum separately on the slide as directed by the manufacturer, 0.05 ml. of the anti-human globulin serum was added to the sedimented cells in each of the duplicate test tubes and to the control tube. After thorough mixing, the duplicate mixtures were placed on the plastic card and on the slide. The mixture on the slide was warmed on a viewing box and rocked gently for 2 minutes while that on the plastic card was allowed to remain undisturbed for 2-2 1/2 minutes and the excess fluid was drained from the card, as were other plastic card tests. The reactions with the two methods were not significantly different. It was found that adherence to the optimum heavy red cell suspension of 40-50 percent of red cells was more necessary for satisfactory results with the plastic card method than with the warm slide method. When this was maintained, the results obtained by the two methods were the same (Table 14).

TABLE II.

COMPARISON OF ANTI-GLOBULIN TESTS BY PLASTIC CARD AND SLIDE METHODS

Specimen Number	Incomplete Anti-$M_g(b)$	Anti-globulin Test	
		Plastic Card	Slide
31	-	4	4
38*	-	3	4
44	+	4	4
625	-	4	4
912	-	3	3
927	-	4	4
1,030	-	4	4
1,21-	±	4	4
1,31?*	-	2	4
1,30-	-	4	4
Positive Control	4	4	4
Negative Control	-	-	-

* Packed, washed cells diluted with one drop of saline.

DISCUSSION

The attempt to evaluate a method for determining blood groups of the AbO and Rh_o specificities leads one to a consideration of the results in the light of the objective. For the blood typing of large numbers of people for the formation of a living blood bank and for the creation of a centralized file of blood types which would be almost immediately available in case of disaster in any area, the object is determination of the most clinically significant blood groups with the highest degree of accuracy compatible with present knowledge and the reasonable accomplishment of that objective.

It is generally recognized that laboratory methods of blood typing from venous specimens, which may be resorted to repeatedly for rechecking, permit a high degree of accuracy. The methods generally preferred are test tube techniques. The objections to test tube procedures for mass blood typing are usually cited as expense, time consumption, requirement of more apparatus and equipment, and the danger of clerical errors. In contrast, the slide test methods are less expensive and the results may be determined immediately, but great objection to the slide method has arisen as a result of inaccuracies that occurred in blood typing by slide method in World War II.

In the present study the determination of AbO and $Rh_o(D)$ blood groups by the plastic card method, a modification of the slide technique, exhibited a technical error of 0.15 percent and a total error of 0.49

percent, when inconclusive results and clerical errors were included, as compared with results found by a laboratory test tube method. The laboratory method involved the additional use of agglutinin determinations, or reverse typings, in the ABO system and the use of multiple antiserums and Coombs anti-globulin testing in the Rh system. With the test tube methods there was one known error which was due to incorrect identification of one ABO blood group in the 9,653 blood samples, an error of 0.01 percent. These results appear to be the reverse of those reported by Allen, Diamond, and Madden (36) in a study of the warm slide method compared with the tube test methods for blood typing using a small series of bloods. It is evident that the plastic card method reported herein would have appeared even more accurate had it been compared with a less comprehensive method of tube testing.

The accuracy of the plastic card method for ABO grouping, even without recourse to reverse typing, was well illustrated in this series. The one error in ABO grouping was due to an inadequate plastic card test, as was readily demonstrable by repetition of the test. Such an error in any method can only be avoided by critical and painstaking attention to detail. The permanence of the plastic card determination readily revealed the nature of the error.

It is possible that factors contributing to the slide testing in ABO blood grouping early in World War II were multiple. The present high standards of potency for blood grouping serums were first proposed by the National Institutes of Health in 1946. The commercial serums now available must possess an adequate minimum of potency against the

subgroups of A as well as against the A and B antigens. The subgroups of A, which are the most difficult to detect of the ABO group, could have been readily overlooked in the blood typing as it was performed during the war with no definite time requirements for the test. The presence of the more critical Rh determination in the series of tests imposes a time requirement on the testing which is advantageous.

An advantage of the test tube method in any serological testing lies in the fact that results can be re-examined over a longer period of time than is usually possible when slide techniques are used, and, therefore, more adequate control of clerical error can be incorporated. In this respect, since the result on the plastic card is not further altered by drying or aging, the plastic card method possesses remarkable possibilities for the control of clerical error. In average slide testing this error is probably unknown. In this study an error of 0.03 percent occurred where three incorrect recordings were made of the results that were evident on the card. This illustrates the value of the permanent record, and it indicates the necessity for a subsequent critical review of the technical and clerical accuracy before an immediately recorded result be used as a permanent donor card or be given to the donor upon completion of the test.

It is known that any one Rh test may fail to identify some of the weakly reacting D^A variants of the Rh positive group. In this series nine bloods, or 0.1 percent, were considered to be of this group. It must be appreciated that the number of bloods placed in this category is largely dependent on the sensitivity and accuracy of the tests used

and on the alertness and experience of persons performing the tests. In addition, it is necessary to determine that the antiserums used do not contain traces of antibodies other than the required anti-Rh_o(D) since antiserums produced by immunization of human subjects, as are those in current use, may contain traces of infrequently occurring antibodies despite careful absorption. Individuals capable of producing antibodies may produce antibodies against many blood group antigens, some of which are undoubtedly still unknown as can be surmised by the increasing list of well characterized independent systems (41) and numerous others of rare occurrence. It follows that the Coombs anti-globulin test could on occasion cause the agglutination of cells sensitized by an unknown antibody since it is not group specific but is specific only for human globulin.

In addition to the routine use of multiple serums on all bloods found to be Rho negative, 10 which were rh'(C) positive were tested with the anti-globulin test since the weak D^u antigen is frequently associated with the rh'(C) antigen. In another series of 22,14 consecutive blood tests the author found 21 percent of those which would have been considered rh'(C) bloods to be $Rh_o(D^u)$ positive by the Coombs anti-globulin test. Henson (42) found 32 percent of a series of bloods originally typed as rh'(C) to be Rh_o'(CD), and Rosenfield et al. (19) found 44.7 percent of bloods originally typed as rh'(C) to be Rh_o'(CD) bloods.

Twenty-two specimens, or 0.22 percent of the bloods tested in this study, were extensively studied with multiple serums and anti-globulin

tests to prove or confirm their classification at $Rh_o(D)$ positive. All these bloods might have been considered low grade D^u, or even Rh_o negative, had they been tested by less sensitive methods and the results would have thus compared even more closely with those of the card test.

It is interesting that of the bloods studied because of discrepant results or doubtful plastic card tests and classified by laboratory testing as weakly reacting $Rh_o(D^u)$ positive bloods, eight were rh'(C) positive. An additional three bloods were $Rh_o(D)$ negative, rh'(C) positive. None was rh''(E) positive. It is perhaps more significant that each of three bloods which were originally typed by the card method as $Rh_o(D)$ positive but considered negative on repeat determination had one doubtfully positive test among the battery of tube tests used. None of these bloods was positive with the Coombs anti-globulin test following attempted sensitization of the cells with five different serums. They were therefore considered $Rh_o(D)$ negative. It is entirely possible that the use of additional anti-human globulin serums might have altered the classification of these tests. It has since been observed that differences are apparent in well standardized anti-human globulin serums. Van Loghem (73) recently demonstrated that a definite prozone phenomenon is exhibited more readily in the tube method than when the thoroughly washed cells in heavy suspension are combined with the anti-human globulin on a slide. It would be of interest to retest these bloods with several anti-human globulin serums.

The plastic card method for the determination of ABO and Rh blood groups was found in this study to yield highly accurate results. It must be performed with meticulous attention to detail under the supervision

of persons well cognizant of its limitations. It yielded a small number of inconclusive results with bloods which require more than a single test to determine the correct Rh group. The plastic card method requires supplementary tests to detect this group of bloods.

SUMMARY

A preliminary study of the plastic card method for the determination of ABO and Rh blood groups of 8,691 individuals was performed under field conditions with average supervision, and the results were compared with the results obtained with bloods from the same individuals by standard laboratory procedures. An analysis of the data indicated the potentialities of the plastic card method and sources of error in the method.

A second study was conducted in the field under re-defined conditions. The blood groups of 9,653 individuals were determined by the plastic card method, and the results were compared with those independently obtained by laboratory tube methods. When discrepancies existed between the results, the laboratory blood specimens were subjected to more extensive study of the blood groups and second blood specimens were obtained from the donors whenever possible.

In the final study a technical error of 0.16 percent and a total error of 0.49 percent, when inconclusive results and clerical errors were included, was found in the plastic card method for the determination of ABO and Rh blood groups as compared with standard tube methods.

The effect of red cell, antiserum, and albumin diluent concentration and the physical variables of time, temperature, and admixture on the results of the plastic card test was studied in a laboratory investigation of the limitations of the method.

The plastic card was examined as a medium for Rh antibody determination, Coombs anti-globulin testing, and study of weakly reacting Rh(D^u) antigens. When the results were compared with results obtained both by standard tube and warm slide methods, it was found that the plastic card method was somewhat less sensitive than the tube methods but approximately comparable to the warm slide method both for antibody titration and for anti-globulin testing. However, the plastic card was unsatisfactory for the detection of weakly reacting $rh_o(D^u)$ antigens.

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