Panel Discussion

The Minor Crossmatch

THE FIELD *of blood transfusion is not unique in being blessed with an abundance of unanswered and controversial problems. Periodically we plan to use these pages for presenting panel discussions on selected topics by authorities who have accepted the editor's invitation "to stick their necks out." Readers' comments and suggestions will be welcomed.*

To do or not to do a minor crossmatch is a disputed matter and will have to be settled some day. We hope the discussion by our panelists will serve to this end.

The Panel

IVOR DUNSFORD ..... Sheffield
ELOISE R. GIBLETT ..... Seattle
JAMES F. MOHN ..... Buffalo
B. P. L. MOORE ..... Toronto
KURT STERN ..... Chicago

1. *Do you believe that it is essential to perform a minor crossmatch?*

Dr. Dunsford: No, providing that (a) the ABO groups of both donor and patient are determined by examination of sera and cells and preferably independently by two technicians, (b) the method employed will detect all known sub-groups of A and B, *i.e.,* the routine use of O serum and O cells and microscopically reading, and (c) the method includes a sensitive antibody screening test.

Dr. Giblett: I believe it is desirable to perform a minor crossmatch as a check on the ABO group of the donor and recipient, and to detect polyagglutinability of the recipient's red cells.

Dr. Mohn: No. It has been stated that antibodies in donor plasmas, other than those of the ABO system, are an almost unknown cause of hemolytic transfusion reactions.[1] In a survey of the medical literature between 1948 and 1955 only two reports were found of hemolytic reactions resulting from irregular isoantibodies both following transfusion of anti-T antibodies into recipients whose erythrocytes exhibited polyagglutinability.[2] Many advocates of the minor crossmatch utilize it in lieu of routinely carrying out a serum check of the ABO group of the donor. There is no justification for substituting this test for a complete determination of ABO groups by both cellular and serum properties.

Dr. Moore: As far as transfusions of homologous group are concerned, the compatibility test used and recommended by this Service has never included a test on the donor serum or plasma. To us, the term "minor-match" denotes no more than a check on the donor isoagglutinin *titer* before certain transfusions of heterologous group, because our concept of a compatibility test holds that: (1) Detection of errors in the donor ABO group is within the compass of a good compatibility routine. (Errors in the recipient's ABO group will not always be detected in this way; but greater care in grouping and the use of Cell and Serum Tube Tests will minimize the chance of error.) (2) Detection of auto-antibodies is the function of the auto-agglutinin controls, and when time permits, of a direct Coombs' test on the recipient's cells. (3) The detection of irregular donor antibodies should have been done at an earlier stage.

2. *Have you encountered any instances in which the minor crossmatch was instrumental in averting a transfusion accident?*

Dr. Dunsford: No. This question was posed to several colleagues in different laboratories and although none uses the minor compatibility test, they know of no reaction that could have been prevented by its use.

Dr. Giblett: We have encountered two cases in which the recipient's red cells were strongly agglutinated by the serum of a large percentage of donors. Although it is difficult to be certain that such polyagglutinability will result in significant *in vivo* red cell destruction by anti-T antibodies in the donor plasma, one is reluctant to expose the patient to such a hazard. A severe hemolytic episode, apparently due to anti-T antibodies in transfused blood, has been described in two infants with polyagglutinable cells.[2] Thus the potentiality of erythrocyte damage, even in adults, certainly exists.

Dr. Mohn: No. The present blood bank organization and transfusion service at the university-affiliated general hospital with which I

am associated was established in 1941. From 1942 through 1960 a total of 76,380 pints of whole blood has been transfused in this institution without employing a minor crossmatch in any case. During this period there were no transfusion accidents recorded which might have been prevented if the minor crossmatch had been included as a part of the routine pre-transfusion testing procedure.

Dr. Moore: Our file of reported reactions gives no indication that any of them could be attributed to the presence of an irregular donor antibody.

Authentic cases of reactions due to donor isoagglutinins are hard to find; we know of none apart from several possible cases in infants following exchange transfusion with group O blood.

3. *If you recommend retaining the minor side of the routine crossmatch, outline the manner in which it is done in your laboratory.*

Dr. Dunsford: It is not recommended. If it were, a saline, albumin and antiglobulin technic would be advised. Furthermore, if the minor compatibility test for blood were recommended, then the recommendations would also have to apply to plasma transfusions. One advantage that could be claimed for performing the minor compatibility test by the antiglobulin technic is that it would reveal cases of acquired hemolytic anemia. The number of cases detected in this manner would not justify the routine application of an antiglobulin minor compatibility test and in any case, surely a patient who has an anemia (the cause of which is unknown or in doubt), should have as part of the routine tests, a direct antiglobulin test before transfusion is considered.

Dr. Giblett: Since the donor's serum has already been screened for antibodies, our minor crossmatch is designed primarily to demonstrate agglutination by anti-A, anti-B and anti-T. Therefore, the procedure is carried out on a slide at room temperature with a drop of donor serum and a drop of serum-suspended recipient cells. The mixture is observed microscopically after two minutes and again after twenty minutes (avoiding drying-out with a Petri dish cover).

Dr. Mohn: No comment.

Dr. Moore: Until about six years ago we tested serial dilutions of donor serum against the recipient's cells when transfusions of heterologous group were necessary; the maximum acceptable titers by a sedimentation technic were 1/128 for adults, 1/16 for infants. Since that time, however, heterologous group transfusions have become infrequent, and we have not deemed a "minor-match" (our connotation) necessary for adult recipients. On the rare occasions when such a transfusion is essential, it seems adequate to match O "hemolysin-free" whole blood (or preferably sedimented cells) for A or B recipients, and A of the same subgroup for AB recipients. In the case of infants, the situation is a little different; here sedimented or packed cells are ideal, otherwise O "hemolysin-free" blood with a plasma titer of less than 1/30 against the infant's cells at 20 C. (sedimentation technic) should be chosen.

4. *It has been suggested that, if the sera of all donors are screened for the presence of antibodies, the minor crossmatch can safely be eliminated. Outline your views in regard to the merits or drawbacks of this proposal.*

Dr. Dunsford: All donor bloods should be routinely screened for antibodies. This achieves: (a) avoiding the issuing of blood which might be potentially dangerous providing the screening test is sufficiently sensitive and the red cells used contain all of the major antigens; (b) on future donations the collection of the blood for diagnostic serum (even comparatively low titer antibodies can be used as a diluent for the stronger anti-sera); (c) in addition, a suitable endorsed blood group card can be issued and with it a letter to the donor stressing the importance of the findings should they ever become hospital patients or in the case of women of child-bearing age, the importance of blood tests in pregnancy. This is a service to the donor and to other laboratory staff who may have to prepare blood for the donor as a patient or her baby at some future date.

Dr. Giblett: Screening of donor serum does not eliminate the possibility of ABO typing errors nor does it have any association with the detection of aberrant characteristics of the recipient's red cells. For this reason, we perform both procedures (*i.e.,* minor crossmatch and screening of donor serum).

Dr. Mohn: Routine screening of donor sera against test cells containing the essential blood group antigens by all of the serologic procedures necessary to detect potentially destructive antibodies can involve a considerable amount of additional work and no little expense to a blood bank. Only the larger blood banks may have their own readily accessible sources of standard red cells containing the most impor-

tant blood group antigens and only those staffed with personnel thoroughly trained in blood group immunology are usually sufficiently experienced in all of the technics required for the detection of the various serologic varieties of blood group antibodies which may occur in the sera of donors. The currently available pools of red cells from commercial sources may not always prove to be satisfactory for this purpose. The incidence of irregular blood group antibodies in the sera of a routine donor population would be one of the important indications for the need to perform routine screening. A recent survey of large community or regional donor centers in this country and Great Britain brought forth statistics indicating an over-all frequency of such antibodies ranging from 0.15 per cent to 0.4 per cent.[3] Comments were received from two British centers where only the sera of unselected Rh negative or of female Rh negative donors were examinted for anti-D antibodies, the rates being 0.57 per cent and 2.0 per cent respectively. The latter figure was felt by its observer to have been an inflated one based on the fact that the immunized women detected by the prenatal testing service in his blood bank were invited to become regular donors. For the smaller hospital blood bank, therefore, the additional technical procedures involved in the detection of such antibodies in only a very small segment of the donor population is of questionable value if their performance interferes with the appropriate concentration on the conduct of accurate blood group determinations and reliable comprehensive compatibility tests on the major side.

Dr. Moore: It is generally accepted that a screening test of donor sera for irregular antibodies should be done; the point in question is where it should be done. In our opinion such a test should form an integral part of the initial processing, whether the laboratory be in a regional center or hospital blood bank. Two reasons can be given: firstly, Rh negative blood should be labeled as such only when it does not contain Rh antibodies; secondly, the initial processing of blood should include every practicable test and check so that at a later stage the technician is free to concentrate upon the basic issues of the compatibility test.

Although most of the antibodies found by the screen-test are of no clinical significance, and determination of their specificity and titer is time-consuming, the occasional discovery of high-titer antibody is sufficiently rewarding. Many of the antibodies found in this way are valuable for other laboratory purposes.

5. *Describe briefly the methods which you recommend for routine screening of donor sera.*

Dr. Dunsford: As part of the ABO group, one volume of the donor's serum is tested against one volume of a 2-5 per cent suspension in saline of pooled A cells and pooled B cells (at least six to each pool). The cell serum mixtures are left for two hours at 18-20 C. and then a portion is transferred to a slide and read microscopically. Reactions of any degree, which are unexpected from the red cell antigens, are an indication for further antibody investigations.

In addition, the sera of all donors are tested against papainized pooled O cells—pooled to give as many antigens as possible. It is a modification of the method described by Stapleton and Moore (J. Lab. Clin. Med. 54: 640, 1959). All Rhesus negative bloods are further checked for anti-Kell by testing the serum against O, rr, Kell positive cells in AB serum. The papain technic has proved very sensitive. Recently an anti-K^b (Cellano)* was detected by this method. (Lang, B. and T. Lodge: Vox Sang. To be published.)

Dr. Giblett: A pool of cells obtained from no more than four (preferably two or three) individuals is selected for the content of antigens, and is pre-tested with weak antibodies of known specificity. If the purpose of the screen is to detect as many antibodies as possible, a saline suspension of cells should be incubated with the serum at three temperatures (cold, room temperature and 37 C.) in succession, necessitating only one tube per serum. Otherwise, incubation at 37 C., followed by an antiglobulin test, is adequate for detecting most incomplete antibodies. An alternative method, which is rapid, less expensive, and particularly useful for (most) Rh antibodies, utilizes either papain (modified by Löws technic) or bromelin. In this instance, the enzyme solution is added to the serum prior to the addition of the red cells. Following incubation at 37 C. for ten to fifteen minutes, the tubes are centrifuged and examined for red cell agglutination. Some antibodies evade detection when enzymes are employed, but it is doubtful that these can be considered hazardous when present in the donor's plasma.

Dr. Mohn: No comment.

Dr. Moore: Most of our regional centers use trypsinized or ficinized cells, but at least one

* See Dunsford, *et al.*: Vox Sang. 4: 148, 1959.—Ed.

prefers a one-stage method employing activated papain. The cells used should be DCe/dce and DcE/dce—one of which is Le (a+)—mixed in equal quantities; the results are read macroscopically after 60 minutes incubation at 37 C. If the volume of work was less, the indirect antiglobulin method would be used with selected cells in order to detect a greater variety of antibodies. For cases requiring the use of an extracorporeal circulation during surgery, the advice of Allen and his colleagues is recommended.[1] A pool of fresh, not inactivated, serum from 5-6 donors is tested against selected cells by the indirect antiglobulin method in addition to the enzyme test just mentioned. The important point in this simplified procedure is to ensure that "the amount of any individual antibody in the test is what it would be if each serum were tested separately."[1]

6. *Is there any significant hazard in transfusing incompatible antibodies in the donor's plasma?*

Dr. Dunsford: Apart from O plasma into not group O recipients, the only documented evidence to suggest transfused antibodies could be dangerous is the experimental work of Jennings and Hindmarsh (Am. J. Clin. Path. **30:** 302, 1958.)

In addition, we know of a case where a baby suffering from hemolytic disease of the newborn due to anti-D was inadvertently transfused with Rhesus negative blood with anti-D present in the plasma. The degree of jaundice was so increased that further replacement transfusions were necessary.

Dr. Giblett: I believe that the hazard exists, but is small. The recent studies of Mohn and his associates[1] have shown that unusually potent anti-D antibodies can cause a severe hemolytic process in Rh positive recipients. However, Rh negative blood is rarely given to Rh positive patients, except in the exchange transfusion of infants.

It is possible that other high-potency antibodies, particularly anti-K, anti-E and anti-c may be capable of similar massive destruction of recipient red cells which contain the appropriate antigen. One may reasonably assume that this risk is most applicable to infants and small children, whose plasma volume is insufficient to dilute the offending antibody. In the event that two or more pints of whole blood are pooled (*e.g.* for cardiac surgery), incomplete antibodies present in one donor can combine with their corresponding antigens from the other donor(s), rendering those cells more susceptible to destruction *in vivo*. Obviously, the extent of that destruction is dependent upon such factors as antibody titer, number of vulnerable red cells, size of the pool, etc.

Dr. Mohn: The results of a study of experimental transfusion of donor plasmas containing blood group antibodies into incompatible normal human recipients have recently been reported.[3] In this investigation, each recipient received an entire unit of ACD plasma selected from donors with antibody titers greater than the probable *in vivo* dilution of a single unit in a recipient's total blood volume. These transfusions included examples of anti-Rh, anti-K and anti-M antibodies of varying titers and representative of different serologic varieties based on their *in vitro* characterization. Although in all volunteers isosensitization of their erythrocytes was deliberately created, no clinical reactions were encountered and no good evidence obtained for any significant loss of red cell mass. These observations indicated that the variety and potency of the warm, incomplete blood group isoantibodies investigated had no measurable deleterious effect on the circulating erythrocytes of the recipients transfused with the equivalent of a single unit of whole blood. There is no reason to believe that these results are not applicable to transfusion therapy of ill patients, provided no intrinsic erythrocyte defect exists which would be the situation with the majority of patients requiring whole blood transfusions in any hospital.

Dr. Moore: If one excludes the obvious danger from ABO incompatibility, the principal remaining risk concerns the transfusion of anti-D to Rh positive recipients. This subject has been studied by Jennings and Hindmarsh[5] and by Mohn and his colleagues;[2, 7] Giblett[4] has briefly reviewed the risks and outlined those cases to whom Rh negative blood containing anti-Rh should not be given. It would appear from this work that a frank hemolytic reaction is most unlikely, although a transient hemolytic anemia may develop. Other high-titer antibodies may be presumed to have a similar effect.

7. *If the minor crossmatch is not used, the donor's pilot sample may be collected in ACD solution. Is there any advantage to using ACD blood instead of a clotted sample?*

Dr. Dunsford: Experimental work carried out in these laboratories in 1948 showed beyond any doubt that the red cell antigens D, C, E, c and Kell were preserved for much longer

periods in ACD mixture than when clotted pilot tubes are used. This work was prompted by the missing of an incompatibility due to anti-Kell using a clotted pilot tube that was six days old. If clotted pilot tubes are used, incompatibilities can be missed by the most senstitive technic when the red cells are beyond a certain age. This would suggest a time limit on clotted samples is essential.

Furthermore, with the use of a wide spectrum antiglobulin reagent, far more difficulties are encountered with clotted pilot tubes due to cold incomplete anti-H than are found with cells in ACD mixture.

Dr. Giblett: Red cells suspended in ACD solution appear to retain their antigenic properties (especially K, Jk^a and Fy^a) longer than the cells present in a clot. However, if ACD-suspended donor cells are used, they must be thoroughly washed before incubating them with the patient's serum, for two reasons: 1. Plasma adhering to the cells can form fibrin strands or clots upon exposure to serum. Cells captured in the fibrin often appear to be agglutinated. 2. ACD, like all anticoagulants, is anti-complementary. If the recipient's serum contains an incomplete antibody which requires complement for its detection by antiglobulin serum, inactivation of that complement may make the antibody undetectable.

Dr. Mohn: In December 1959 our routine pretransfusion testing technics were altered to include the collection of pilot samples of donor blood in ACD solution exclusively for use in compatibility testing. This has been accomplished through the use of sealed and numbered segments of the plastic donor tube (Fenwal). During 1960, 13,100 compatibility tests were carried out with donor cells preserved in this manner. This has turned out to be a highly satisfactory procedure making available donor erythrocytes in as excellent condition as those in the unit throughout the dating period of the blood itself. No longer do we encounter those occasionally difficult to interpret crossmatching tests with donor bloods very near to the twenty-one day expiration date where considerable hemolysis has occurred in the clotted pilot sample and pseudoagglutination or slimy, stringing together of the donor cells is observed in the test. This procedure has also almost eliminated any clerical error in identification of the donor samples used in the laboratory in the crossmatch since they are integral parts of the plastic donor unit itself until removed and bear the number of the unit. A single clotted specimen is still obtained only for examining for the absence of serologic evidences for syphilis and for performing the routine ABO and Rh grouping determinations on the donors.

Dr. Moore: The pilot tubes used in our Service have always contained ACD. There are two important reasons for this choice: (1) Compatibility tests are of limited value if the agglutinability of the donor cells is impaired. Most blood group antigens are still quite effective after 14-21 days in ACD, the main exceptions being c and P.[5] Crawford, Cutbush and Mollison,[3] on the other hand, have shown that after even seven days at 4 C. the reactions of cells from clotted blood may be distinctly weaker than those of fresh cells. (2) If washed cells from citrated blood are used in the compatibility test, it is then possible to use antiglobulin reagents containing potent "anti-complement" components without fear of difficulties from weak false-positive reactions.

It seems to me that the need for ACD in pilot tubes is a fundamental issue and should take precedence over such disputed questions as the need for "minor-match."

8. *Some institutions screen the serum of each patient for the presence of antibodies at the time of admission in order to uncover crossmatching problems in advance. Would you favor widespread adoption of this procedure?*

Dr. Dunsford: Yes. This divides into two parts—the emergency admission and the planned case.

In the emergency admission, a quick screen with a pool of cells containing as many antigens as possible by a sensitive antiglobulin technic should be carried out, or at least by an enzyme or albumin technic. This enables the technician to set up the major compatibility test against more donor bloods than those requested in the hope of finding sufficient for the immediate need.

In the planned case, the antibody detection screen and subsequent identification should be routine to enable coordination of the services for the supply of special typed blood. This planning leads to better feelings between the suppliers and users of the blood and enables better service to the patient and our colleagues in other hospitals (see answer to question 4—para. c). In fact, the findings of immune antibodies may be a contra-indication to further transfusions if these can be avoided.

In our own laboratories a Regional register of known immunized patients or would-be patients is maintained and is proving invalu-

able. Since we also maintain the Regional panel of typed donor bloods, it leads to greater efficiency.

Dr. Giblett: The procedure is not possible in this area, because all of the typing and compatibility tests are performed in the laboratory of one central blood bank. It is our practice to screen the patient's serum at the time of the crossmatch, the procedures being carried out in parallel.

There are obvious advantages in having advance information about the presence of antibodies in a patient's serum. However, it would be unfortunate if a negative "screen" were considered sufficient reason for omitting a careful crossmatch. Screening technics are not infallible, and there is always the possibility that the recipient has an antibody with a corresponding antigen not represented in the screening cells.

Dr. Mohn: I do not favor widespread adoption of this procedure for most of the reasons given for my negative reaction to routine screening of donor sera. Again this would require ready availability of well preserved individual cells or pools of cells containing all of the important erythrocyte antigens. To the smaller hospital blood bank the time and expense involved in carrying this out on a regular basis might be prohibitive. This objection could be overcome in part by limiting such screening to the sera of patients found on admission to have a known history of multiple transfusions during a previous admission in the same or some other institution and likely to be candidates for transfusion again. This would depend upon accurate information of this nature being obtained and transmitted promptly to the blood bank well in advance of any request for a blood transfusion.

Dr. Moore: Some years ago those of our centers that offer an area crossmatching program were recommended to use an indirect antiglobulin screen test of the recipient's serum against a mixture of no more than two fresh cell-samples containing between them the antigens S, D, C, E, c, K, Le^a, Fy^a, and Jk^a. Apart from uncovering a fruitful source of interesting antibodies that are bystanders so far as the donor antigens are concerned, this step was intended to control the possibility that certain donor antigens from 14-21 day blood might not be effective in detecting a weak antibody in the recipient.[6] We also recommended that the serum of cases requiring periodic transfusion should be investigated from time to time for irregular antibodies, so that emergency transfusions need not be delayed or withheld for lack of compatible blood. I doubt whether it would be practicable to perform an antibody screen test on all patients at the time of admission in any but the smallest centers. However it is a sound idea to do this test before the compatibility routine. I know of several Canadian hospitals that feel such a course is an essential safety factor as well as an invaluable source of antibodies for test purposes.

Dr. Stern: The minor crossmatch is an integral, valuable, and readily performed part of pretransfusion tests which serve as safeguards to protect the recipient from some of the most serious hazards of transfusion therapy. Reasons that can be adduced in justification of this statement can be divided into three categories. The *first,* and most important one, concerns *detection of ABO incompatibility.* In this respect the minor crossmatch is a check procedure analogous to the "reverse" or "confirmation" grouping which is generally considered to be essential for determination of the ABO group. Accordingly, the minor crossmatch may reveal technical or clerical errors in ABO groups of donor and prospective recipient that otherwise might escape detection. Jennings and Hindmarsh[9] have pointed out that errors in ABO group detected *only* by the minor crossmatch represent situations in which the blood selected may be considered as "universal donor" or the patient as "universal recipient." However, this does not abolish the significance of such errors, especially since they may not only affect the donor and patient directly involved but, in some instances, may be even of more serious import for utilization of other units of blood or samples of other prospective recipients if the error has resulted from confusion of specimens or similar forms of mistaken identity. Furthermore, in some situations blood incompatible on the major as well as on the minor side may have been selected erroneously, *e.g.,* A blood for a B recipient, or *vice versa,* but the major crossmatch may fail to reveal the incompatibility for one or more of the following reasons: isoagglutinins in the recipient may be of extremely low titer, such as in some patients with leukemias or lymphomas,[5, 11] hypogammaglobulinemia or agammaglobulinemia;[2] or, the reactivity of the donor's red cells may be poor because of age of the pilot tube, inadequate preservation, or presence of a weakly reacting subgroup of A. In such instances the incompatibility in the minor crossmatch will alert the worker to search for the cause of the unexpected agglutination observed and thus use of mismatched blood will be averted. Finally, the

minor crossmatch may reveal unusual complications, such as presence in the donor's serum of anti-A_1 active at room or even body temperature.

The *second* reason is based on the *obligatory nature* of the minor crossmatch in certain specific situations. If such a need arises as emergency, blood bank workers not attuned to the regular performance of the minor crossmatch may be at a serious disadvantage. The most common cause for mandatory performance of the minor crossmatch is associated with use of "universal donors" or transfusion of "universal recipients" outside of their own ABO groups. Improper selection of blood in these situations has frequently been followed by disastrous consequences.[6, 7, 8] Sometimes an Rh-negative patient may be transfused with Rh-negative blood containing Rh antibodies. If it cannot be excluded that the patient may have received Rh-positive blood within the preceding weeks, it is essential that the minor crossmatch demonstrate the absence of surviving Rh-positive red cells which could react with the transfused antibody. In general, when it is intended to use Rh-negative blood with Rh antibodies for presumably Rh-negative patients, it is desirable to perform a minor crossmatch in order to rule out presence in the recipient's red cells of D^u, rh′ (C), and rh″ (E), all of which might interact with the donor's antibodies. Obviously, such minor crossmatch tests must utilize technics capable of demonstrating presence of incomplete antibodies (antiglobulin or enzyme tests).

The *third,* and possibly least important, reason for doing minor crossmatch tests is *detection of irregular isoantibodies* in the donor serum as shown by reactivity with the recipient's red cells. As a matter of course, detection of such antibodies critically depends on the technic of compatibility tests used; *i.e.,* only with the help of antiglobulin and/or enzyme methods can one expect to demonstrate incomplete antibodies for Kell, Duffy, Kidd and other blood factors. A special problem is represented by donors with "high-frequency" antibodies. While at first glance it might appear as extremely unlikely that a donor with anti-Tj^a (anti-P + anti-P_1) might become a donor for a random recipient, such an incident *almost* occurred within the last year, thus proving again that blood bank work, just as life in general, may be stranger than fiction. Some time ago we had the opportunity of studying a family with six Tj^a-negative siblings.[3] A few months later a telephone inquiry was received from a small hospital, quite a distance from Chicago, where the father of the Tj^a-negative persons was scheduled for surgery. One of his sons, known to us as Tj^a-negative, was thought to be a suitable donor for his father since both were group O Rh-positive. However, to the surprise of the laboratory staff, hemolysis was observed in the minor crossmatch using the father's red cells and the son's serum. For obvious reasons, it was recommended to use another donor for the patient. With the increasing use of hemotherapy and the broadening of the donor population, incidence of isoimmunization may be expected to rise among blood donors, including presence of "high-frequency" antibodies. Another situation of admittedly exceedingly remote probability is that of a recipient with the extremely rare factor Mg; on the other hand, donors who might have the corresponding antibody are not at all uncommon.[1] In this hypothetical situation only the minor crossmatch can detect the incompatibility.

Several arguments are offered by proponents for elimination of the minor crossmatch. Detection of "minor" incompatibilities is said to be unimportant because transfusion of incompatible antibodies is supposedly of little consequence for the recipient. Apart from the fact that many serious, and even some fatal, transfusion reactions have been recorded as a result of use of "dangerous universal donor" blood,[6-8] this reasoning can be subjected to additional criticism. Experimental studies of Jennings and Hindmarsh[9] and of Culp and Chaplin[4] have disclosed evidence for destruction of red cells as a result of transfusion of some incompatible antibodies. It is ovbiously impossible to predict in each case in advance whether and to what extent such effects will be encountered. Most importantly, one must not lose sight of the fact that the goal of hemotherapy is not merely elimination of serious transfusion reactions, but rather assurance of maximum benefit to the patient. This certainly is *not* accomplished when even a small fraction of the red cells of the patient, whose vital functions are often already impaired, is subjected to accelerated destruction.

Neither can this writer agree to the assertion that the minor crossmatch adds too much complexity to blood bank work. Since red cells of the recipient and serum of the donor must be available for other parts of the pretransfusion tests, the preparation of one additional mixture cannot be considered to impose a serious burden on the technologist. There is also no need for appreciably prolonging the time required for

completion of the test since the same periods of incubation can be used for the major and minor crossmatch tests. The fear has also been expressed that the minor crossmatch test may become a potential source of error because the technologist may prepare the wrong mixture of serum and red cells or misinterpret the final readings. This objection does not appear to have much merit since opportunities for similar mistakes are present in connection with "direct" and "reverse" ABO grouping. A technologist prone to such sources of confusion should probably be best diverted from blood bank work to some other less critical activity.

It is difficult to pinpoint concrete incidents in which the minor crossmatch was instrumental in averting transfusion accidents. Jennings and Hindmarsh[9] included in their report the number and types of irregular isoantibodies detected by means of the minor crossmatch test at the Mount Sinai Blood Center, Chicago. Since then three additional donor antibodies were found: an anti-P_1 with good saline and antiglobulin reactivity, and one instance each of low-titered anti-rh″ (E) and anti-Jk^a. None of these units of blood was used for recipients with the corresponding blood factors.[12]

The technic of the minor crossmatch recommended[12] parallels that of the major crossmatch: (a) a 2 per cent suspension in saline of the recipient's red cells is added to the donor's serum and the mixture centrifuged immediately; (b) if this step has failed to produce agglutination, the mixture is incubated for 15 minutes at 37 C.; (c) in the absence of agglutination after the preceding step, the mixture is converted into an antiglobulin test. For investigative purposes the cysteine-papain crossmatch technic[11] has also been used; results of this work will be presented as a separate report. Whenever blood is used outside of its own ABO group, the minor crossmatch test proposed by Grove-Rasmussen, *et al.*,[8] is to be used.

It is of considerable advantage and most desirable to screen serums of donors for antibodies. The critical question is that of the extent to which such screening should and can be done. We have reported on the use of pooled papain-treated red cells for such purposes,[10] a method which detects almost all Rh and some other types of irregular antibodies. It must, however, be kept in mind that in many small blood banks screening technics are not only difficult to institute but may be also insufficiently reliable because of lack of adequate facilities and experienced personnel. Under such conditions I would strongly object to replacement of the minor crossmatch by screening tests of the donors' serums. Obviously there is also the inherent danger that such reasoning may be applied with seemingly equal justification to screening of the patients' serums as a step permitting elimination of the *major* crossmatch.

Opinions on the hazards associated with the transfusion of incompatible antibodies have been expressed in preceding parts of this discussion.

Collection of donor's pilot sample in ACD solution does not seem to me to be predicated on elimination of the minor crossmatch. Serum of the donor is still needed for antibody screening and for most methods of serologic testing for syphilis. If sufficient evidence is available for better preservation of reactivity of hemoantigens in ACD than in clotted blood, it would appear desirable to collect both types of samples of donor blood.

Screening of the serums of prospective recipients of blood transfusions for presence of irregular isoantibodies is most desirable. A method, such as that of pooled papain-treated red cells,[10] appears to be simple enough to be included in routine pretransfusion tests. In addition to detection of Rh antibodies, this method also readily establishes presence of autoantibodies and thus provides information of considerable importance for elimination of technical sources of error in selection of blood as well as valuable guides for the clinical management of the patient.

Editor's Comment

The panelists' opinions concerning the necessity for performing the minor crossmatch vary from an unqualified "No" to a flat "Yes." The decision to retain or to drop this portion of the crossmatch will have to be made on an individual basis. Its major function seems to be to detect errors in ABO grouping. If the patient's and donor's blood groups are performed properly, then the minor crossmatch is considered of minor importance by the majority of the panelists. Dr. Giblett retains it as a simple slide test "to demonstrate agglutination by anti-A, anti-B and anti-T, since the donor's serum has already been screened for antibodies." Instances in which donor's antibodies have caused overt reactions appear to be difficult to find. The merits of screening patient's and donor's sera for antibodies are generally accepted but not deemed universally feasible.

The ACD donor pilot sample is favored over the usual clotted specimen.

You may be right or wrong in doing or not doing the minor crossmatch. The answer depends on what else you are doing and how well you do it.

References

Dr. Giblett:

1. Mohn, J.: Personal communication.
2. Mollison, P. L.: Blood Transfusion in Clinical Medicine, 2nd ed., Oxford, Blackwell Scientific Publications, Ltd., 1956, pp. 262-264, 392.

Dr. Mohn:

1. Mollison, P. L.: Blood Transfusion in Clinical Medicine, 2nd ed., Oxford, Blackwell Scientific Publications, Ltd., 1956, p. 392.
2. Jennings, E. R. and C. Hindmarsh: The significance of the minor crossmatch. Amer. J. Clin. Path. **30:** 302, 1958.
3. Mohn, J. F., R. M. Lambert, H. S. Bowman and F. W. Brason: Experimental transfusion of donor plasma containing blood group antibodies into incompatible normal human recipients. I. Absence of destruction of red cell mass with anti-Rh, anti-Kell and anti-M. Brit. J. Haemat. **7:** 112, 1961.

Dr. Moore:

1. Allen, F. H., Jr., T. VonBercken and S. J. Boyce: Crossmatching of blood for massive transfusion. Bull. Amer. Assoc. Blood Banks. **12:** 267, 1959.
2. Bowman, H. S., F. W. Brason, J. F. Mohn and R. M. Lambert: Induction of iso-immune haemolytic anaemia by a transfusion of plasma containing exceptional anti-CD antibodies. Amer. Assoc. Blood Banks. Annual Meeting 1959.
3. Crawford, H., M. Cutbush and P. L. Mollison: Preservation of red cells for blood grouping tests. Vox Sang. **5:** 149, 1954.
4. Giblett, E. R.: Questions and Answers. Bull. Amer. Assoc. Blood Banks. **13:** 342, 1960.
5. Jennings, E. R. and C. Hindmarsh: The significance of the minor crossmatch. Amer. J. Clin. Path. **30:** 302, 1958.
6. Linins, I., J. McIntyre and B. P. L. Moore: The effect of storage upon the blood group antigens, P, c, K, and Fy^a. Can. J. Med. Tech. **21:** 47, 1959.
7. Mohn, J. F., R. M. Lambert, H. S. Bowman and F. W. Brason: Experimental transfusion of donor plasma containing blood group antibodies into incompatible normal human recipients. Amer. Assoc. Blood Banks. Annual Meeting 1959.

Dr. Stern:

1. Allen, F. H., Jr., P. A. Corcoran, H. B. Kento and N. Brearo: M^g, a new blood group antigen in the MNS system. Vox Sang. **3:** 81, 1958.
2. Brem, T. H. and M. E. Morton: Defective serum gammaglobulin formation. Ann. Int. Med. **43:** 465, 1955.
3. Busch, S. and K. Stern: Report on a family with six Tj^a-negative siblings. In preparation.
4. Culp, N. W. and H. Chaplin, Jr.: The effects of concentrated eluted anti-red blood cell antibodies on the *in vivo* survival of normal red cells. Blood. **15:** 525, 1955.
5. Davidsohn, I.: Isoagglutinin titers in serum disease, in leukemias, in infectious mononucleosis, and after blood transfusions. Am. J. Clin. Path. **8:** 179, 1938.
6. Davidsohn, I. and K. Stern: Diagnosis of hemolytic transfusion reactions. Am. J. Clin. Path. **25:** 381, 1955.
7. Ervin, D. M. and L. E. Young: Dangerous universal donors. I. Observations on destruction of recipient's A cells after transfusion of O blood containing high titer of A antibodies of immune type not easily neutralizable by soluble A substance. Blood. **5:** 61, 1950.
8. Grove-Rasmussen, M., R. S. Shaw and E. Marceaux: Hemolytic transfusion reaction in group A patient receiving group O blood. Am. J. Clin. Path. **25:** 381, 1955.
9. Jennings, E. R. and C. Hindmarsh: The significance of the minor crossmatch. Am. J. Clin. Path. **30:** 302, 1958.
10. Stern, K., S. Busch and A. Buznitsky: A crossmatching test using activated papain. Am. J. Clin. Path. **27:** 707, 1957.
11. Teitelbaum, J. I., J. Wiener and J. E. Desforges: A serologic and electrophoretic study of the malignant and proliferative disorders of the hematopoietic and reticuloendothelial system. J. Lab. Clin. Med. **53:** 535, 1959.
12. Laboratory and clinical data referred to in this discussion reflect the past activity of the author as Director, Blood Center of Mount Sinai Medical Research Foundation, Chicago Illinois.