Compatibility testing without a centrifuge: 
the slide Polybrene method

Marie Lin

BACKGROUND: A simple and rapid slide Polybrene method (SP) for pretransfusion compatibility testing is described. SP is particularly suitable for use in developing countries where, due to limited resources, centrifuges and biologic reagents may not be readily available.

STUDY DESIGN AND METHODS: The original manual Polybrene method (MP) was modified for use on glass microscope slides, eliminating the need for test tubes and centrifugation. The sensitivity of SP for detecting alloantibodies to RBC antigens was compared with that of MP and the IAT.

RESULTS: Both SP and MP were more sensitive than the IAT for detecting anti-E. SP detected 21 of 23 examples of anti-E and 7 of 8 examples of anti-E. Kidd and Diego system antibodies were also readily detectable by SP, although the reactions were weaker than those observed with both MP and IAT. However, both SP and MP failed to detect some examples of antibodies to Kell system antigens.

CONCLUSIONS: SP is an acceptable method for compatibility testing in developing countries, particularly in populations where the frequency of K is low (e.g., southeast Asia). The reagents are inexpensive and can be prepared in-house. SP is simple to use, does not require a centrifuge, and can be performed by personnel with minimal training.

ABBRVIATIONS: MP = manual Polybrene; SP = slide Polybrene.

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Most developed countries have introduced standardized methods for pretransfusion testing that require the use of specific reagents and equipment (e.g., antiglobulin serum, centrifuges, etc.). However, for underdeveloped countries such items may be unavailable or too expensive to purchase. At a recent "Manual Polybrene" Workshop in Laos (December 2000, Lao Red Cross Blood Center, Vientiane) and an "Immunohematology in Taiwan" Workshop in Vietnam (December 2002, Viet-Duc University Hospital, Hanoi), it was discovered that very few centrifuges were available for use in the transfusion services of these countries. Therefore, the development of a compatibility testing procedure that did not require the use of a centrifuge or expensive reagents was of particular importance.

Past experience in developing a national blood program for Taiwan in the 1980s demonstrated the importance of having a simple, rapid, and inexpensive method for compatibility testing to standardize pretransfusion testing procedures. Before 1980, transfusion medicine was an area of low priority in Taiwan and only a few teaching hospital blood banks had adequate equipment, finance, and staff to incorporate the more expensive and time-consuming standard Western procedures. However, in 1983 the manual Polybrene (MP) method, which is a simple and rapid procedure for the detection of RBC alloantibodies, was introduced at Mackay Memorial Hospital. Within a few years, MP was incorporated successfully into routine pretransfusion testing procedures throughout the whole of Taiwan (including both large and small institutions).

In this report, the slide Polybrene (SP) method, which is even simpler to perform than MP, is described. The reagents required for the SP method are inexpensive and can be prepared in-house. In addition, blood-bank personnel require only minimal training to perform the test satisfactorily, and the use of a centrifuge is not required. SP also demonstrates good sensitivity for the detection of all antibodies that are of clinical significance in southeast Asia.
MATERIALS AND METHODS

Reagents
Low-ionic-strength medium was prepared as for the MP method described by Lalezari and Jiang. A 10-percent stock solution of Polybrene (hexadimethrine bromide, Sigma Chemical, St. Louis, MO) in normal saline was also prepared as described by Lalezari and Jiang. A 0.1-percent working Polybrene solution was prepared by appropriate dilution of the 10-percent stock solution in saline.

Resuspending solution for reversing the nonspecific Polybrene induced aggregation was prepared by mixing 60 mL 0.4 mol/L trisodium citrate solution and 40 mL 5-percent dextrose. (Note: the working Polybrene solution and trisodium citrate concentrations used in SP were double the concentrations that were used in MP.)

SP method
Sensitization phase. An ellipse, 3 × 1.5 cm in size, is drawn on a glass microscope slide with a wax pencil to prevent overflowing of reagents. Three drops of low-ionic-strength medium are added to the slide followed by 2 drops of test serum (or plasma) and 1 drop (50 μL) of 20 percent RBCs in saline (or 10 μL packed RBCs). The reagents are mixed thoroughly with an applicator stick and incubated at room temperature (about 22°C) for 1 minute.

Polybrene aggregation phase. One drop of the 0.1-percent Polybrene working solution is then added to the reagents on the slide, mixed with an applicator stick, and incubated at room temperature for 1 minute. RBC aggregation usually begins to appear about 30 seconds after the addition of the 0.1-percent Polybrene solution and is complete within 1 minute.

Resuspension phase. One drop of resuspending solution is added, and the slide is gently rocked by hand for about 10 seconds until any nonspecific Polybrene-induced aggregation has dispersed. True antibody-induced agglutination does not dissociate and can be readily observed macroscopically. For weaker reactions, the agglutinates can be read using a magnifier. Results should be evaluated as soon as possible, and certainly no later than 3 minutes after the resuspending solution has been added.

Controls. Daily quality controls should include a weakly reacting anti-E or anti-D as a positive control and inert AB serum as a negative control.

MP method
MP was performed as described by Lalezari and Jiang except that no supplementary antiglobulin phase was performed. Tests were examined microscopically.

IAT
A standard saline IAT was performed by incubating at 37°C for 30 minutes, followed by washing and the addition of antihuman IgG (Gamma Biologicals, Houston, TX). Tests were examined microscopically.

RESULTS
A comparison of the sensitivities of SP, MP, and IAT for the detection of various alloantibodies are shown in Tables 1 and 2.

From Table 1, it can be seen that SP and MP are more sensitive than IAT for the detection of anti-E. However, IAT is more sensitive than both SP and MP for the detection of anti- "Mi"a, -K, -Jk"a, and -Jk"b.

From Table 2, it can be seen that SP detected 21 of 23 examples of anti-"Mi"a and 7 of 8 examples of anti-E. More significantly, SP readily detected all ABO incompatibilities (Table 2). SP also readily detected other important alloan-
tibodies of clinical significance, including antibodies against antigens of the Kidd and Diego blood group systems, although reactions were weaker than those observed with MP and IAT.

Among 85 antibodies from patients that were tested, 74 antibodies were detectable by SP, including 10 anti-A and 10 anti-B. Anti-Kp\(^a\) could only be detected by IAT (Table 2) and higher anti-K titers were obtained by IAT than by both SP and MP (Table 1). Therefore, the main disadvantage of the two Polybrene methods is that a small number of antibodies of the Kell blood group system will not be detected. However, because the frequency of K in oriental populations is very low, this is not clinically significant.

### DISCUSSION

Slide methods have generally been considered inferior to tube methods with regards the detection of clinically significant alloantibodies. This is mainly due to the fact that in tube methods, RBCs are forced close together by centrifugal force, which thus enhances hemagglutination. However, in the SP method RBCs are brought close together by the action of the positively charged 0.1-percent Polybrene reagent, resulting in nonspecific RBC aggregation. Heparin interferes with the test, and 2 to 3 times of Polybrene should be added if heparinized samples are used (i.e., 2 or 3 drops of Polybrene). The Polybrene-induced aggregation can be quickly reversed by adding 1 drop of 0.4 mol/L citrate resuspending solution leaving any specific antibody-induced agglutination intact.

In this study, the sensitivity and efficacy of SP in detecting alloantibodies were compared simultaneously with MP and IAT by testing antibodies that were encountered during antibody screening and cross-matching in the Blood Bank, Mackay Memorial Hospital; antibodies obtained from the “Serum, Cells and Rare Fluids International Immunohematology Exchange Group” (SCARF), which included antibodies rarely found in Taiwan; two highly diluted commercial MoAb (anti-D and anti-E), which were routinely used as daily positive controls for SP and MP; and also several commercial antisera as shown in Table 1. The results show that SP detected most alloantibodies of clinical significance, especially anti-E and anti-Mi\(^a\). These two antibodies are the most common alloantibodies of clinical significance in Taiwan and most likely also in the rest of southeast Asia. Anti-Mi\(^a\) is used in Taiwan to describe antibodies that react with RBCs of the Mi\(\text{III}\) phenotype.\(^6\) Other alloantibodies such as anti-D, -K, -Jk\(^a\), -Jk\(^b\), -Fy\(^a\), and -Fy\(^b\) were also detected by SP with a sensitivity similar to that of MP. Although SP (and MP) are not as sensitive as IAT for the detection of anti-K, because most patients and donors in southeast Asia are K negative, the incidence of anti-K would be expected to be very rare. This is indeed the case, and during the past 20 years in Taiwan, only one antibody against an antigen of the Kell blood group system has been found (anti-Ku in a K\(null\) person). Therefore, the implementation of a sensitive procedure for the detection of antibodies to antigens of the Kell blood group system would appear to be of low priority in routine compatibility procedures for southeast Asia.

SP is extremely rapid (about 5 min), cost effective (reagents for the test can be prepared simply and in-house), and is easy to perform. Personnel require only 1 day’s training to perform the test with confidence. Therefore, the introduction of SP in countries with limited resources, and especially in countries where pretransfusion testing is limited to ABO grouping, will help significantly to improve transfusion safety. In such countries, many antibodies of clinical significance, which until now have been undetected, can now be detected.

Transfusion services in developing countries, not only lack centrifuges but also lack finances for purchasing the reagents and training new staff. In such situations, SP is the method of choice for routine pretransfusion testing so as to improve patient safety.

### ACKNOWLEDGMENTS

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### TABLE 2. Numbers of patients in whom antibodies were detected and methods of detection

<table>
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<th>Anti-</th>
<th>Number of patients</th>
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<th>MP</th>
<th>IAT</th>
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* Bombay phenotype.
REFERENCES

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