Special Article

The Gel Test: Twelve - Year's Experience in Thailand

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Within twelve years of its introduction for blood group serology testing in the blood bank, the gel test has gained widespread usage throughout Thailand, especially for antibody screening, crossmatching, direct antiglobulin testing and blood group antigen typing. The gel test has been proven to be more sensitive than the standard conventional tube test. Furthermore, the test itself is simple and practical for mass screening and application in emergency situations.

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In vitro red blood cell (RBC) antigen-antibody reactions are usually detected by agglutination tests. This two-stage process is affected by several physical and chemical variables, including pH, temperature, ionic strength, time, antigen-antibody ratio, characteristics of the antigen and antibody molecules, and the natural forces that keep the red blood cells apart. Although immunohematological procedures involving hemagglutination are designed to take these conditions into consideration, variations in the techniques used can affect the reliability and reproducibility of the final results.

The lack of consistency in transfer-pipette design and the volumes delivered by them have been well documented[1]. Inherent variations occur when red blood cell suspensions are prepared. Wide-ranging differences exist in techniques to resuspend cell buttons, and overzealous resuspension may disturb the fragile agglutination encountered with weak reactions, particularly in the antiglobulin phase[2]. The washing procedure itself, which is necessary before the addition of antihuman globulin (AHG) reagent, has steps in which faulty or poor techniques may adversely affect the results. The final end point in conventional hemagglutination has limited stability, so that the test results must be interpreted immediately on completion. Due to these problems to obtain good results, the agglutination reactions must be examined by a qualified person within a short space of time.

Although these problems have long been recognized in immunohematology, practical solutions have been in short supply. Until recently, in 1980, Lapierre, et al developed the gel test for use in serological procedures in order to obtain and retain hemagglutination reactions for interpretation in a rapid, simple and standardized manner[3]. A major benefit of this technology is that it obviates the need for a wash phase during antiglobulin testing, thus eliminating many of the potential errors known

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to occur due either to poor washing techniques and subsequent neutralization of antihuman globulin reagent or to over-vigorous handling of the completed test prior to reading. The gel test system utilizes Sephadex™ gels in which after centrifugation, agglutinates are trapped, negative reactions appearing as a discrete cell button at the base of the column and clearly different from the positive ones, which vary depending on their strength as shown in Fig. 1. After research and development with DiaMed AG, Switzerland, the patent gel test system was released in Europe in 1988.

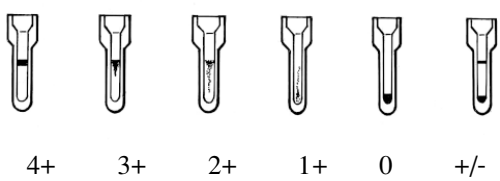

Fig. 1 Gel test agglutination reaction patterns and grading schemes: negative reaction (0) and mixed field agglutination(+/-) are clearly different from positive reactions

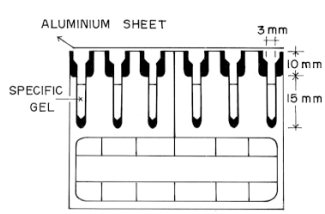

Fig. 2 An example of a card developed for the gel test containing six microtubes. The gels in each microtube may be neutral, or impregnated with specific antisera or AHG

The gel test employs the principle of controlled centrifugation of RBCs through a suitable dextran acrylamide gel contained in a specifically devised microtube that is composed of an upper reaction chamber and a barrel with a conical button. The reaction chamber of the microtube (Fig. 2) is designed to allow prior incubation of test serum and RBCs above the gel if necessary. Six microtubes are held together in a plastic card (Fig 2). Only three types of gel are required to perform the majority of applications in blood group serology. The gels may be neutral, or impregnated with specific antisera or AHG. The density gradient centrifugation principle of this technique ensures that the serum is separated from the reaction mixture and is retained in the chamber at the top of gel column. Antiglobulin testing is therefore undertaken without a wash phase. A neutral gel contains gel in an osmotically balanced buffer and acts only as a trap for agglutinated RBCs. This type of gel is used primarily for direct agglutination tests, such as ABO serum grouping, or antibody screening and identification using enzyme-treated or untreated RBCs. Specific gels contain a mixture of gel and specific antiserum such as anti-A, anti-B and anti-D. The antisera integrated into the specific gels must be carefully selected to ensure that they give strong reactions while they maintain their specificity. These gels are used for antigen typing application. Antiglobulin gels incorporate AHG as an integral component of the gel mixture. These gels are used to perform applications that require antiglobulin tests such as antibody screening or direct antiglobulin testing. Blood bank staff in many routine laboratories both in the USA and throughout Europe, have evaluated this system and have found that its use enhances RBC antibody detection rates, as well as allowing new opportunities for control and checking of complete serological tests[4-11].

In Thailand, the gel test was first introduced at the National Blood Centre, Thai Red Cross Society and the Division of Blood Bank, Department of Pathology, Phramongkuklao Hospital, Bangkok in 1993 and has become popular in several blood banks[12-13]. It has been used in various tests in the blood bank. Several studies have been carried out comparing the gel test and the conventional tube test. The gel test is useful in ABO, Rh and other blood group typing, especially for the selection of appropriate RBCs for multitransfused patients.

Its use is less-time consuming and practical for population screening and application in emergency situations[14,15]. Regarding antibody screening, antibody identification and crossmatching, although the gel test is less sensitive in the detection of cold alloantibodies such as anti-P_1 and antibodies in the Lewis system; it has been proven to be more sensitive than the standard conventional tube test for the detection of clinically significant antibodies, which are commonly found in Thai populations, such as anti-E and anti-Mi^a[16,17]. Moreover, in hematology services, the gel test is used in direct antiglobulin testing and identifying antibodies on the RBCs of patients with thalassemia, autoimmune hemolytic anemia and hemolytic disease of the newborn, with the overall sensitivity and specificity of the test at 93.5% and 88.6%, respectively[18]. Additionally, it has been used in the diagnosis of paroxysmal nocturnal hemoglobinuria (PNH) to detect the PNH RBC populations compared with the standard acid serum test. The sensitivity and specificity were 100%. It was found that the PNH gel test could be used as a screening test, especially in laboratories that are already using this system for blood grouping and antibody detection[19]. Although the cost of the gel test is more expensive than the conventional tube test in antiglobulin testing, for some instances such as the detection of immunoglobulin-coated RBCs by direct antiglobulin testing and typing for Rh and other blood group antigens; the gel test provides more cost effective than the conventional tube test. From our experience the gel test is a highly sensitive system for most serological tests with many advantages.

First, it is easy to read the reactions because no microscope equipment is used. It provides a clear-cut grading system. Grades 4+, 3+, 2+, 1+ and w+ can be distinguished by the naked eyes. The agglutination reactions can be re-read so there is the possibility for a second or repeated reading since the reaction patterns are stable for hours or even for days if the cards are closed with tape and kept in the refrigerator at 4°C. Another benefit is a photocopy of the positive or negative reactions can be made and kept as firm laboratory data. In the cases of mismatch transfusion or ABO subgroups, two cell populations can be distinguished easily by this test. Moreover, since the rouleaux formation could be the cause of a false positive result in the slide or tube tests, it was no cause for discrepancies in the gel test.

Second, the gel test is easy to handle. Dispensing is easier; instead of using the pasteur pipette, the automatic pipettes and the disposable tips are used, which are more accurate and reduce the problem of broken glass or glass cleaning. The removal of washing procedures in the antiglobulin phase has eliminated the formation of aerosols and minimized the risk of contamination by washing fluids. Therefore, the risk of contracting blood-transmitted diseases, such as hepatitis or HIV infection, will decrease. Hence, it requires not only less time but also fewer technical skills, especially in the antiglobulin testing. This will result in a reduction of technical errors associated with the poor washing techniques and/or inappropriate handling of the complete liquid-phase test of the conventional tube test. Because of the reduction in the number test steps, fewer human errors can be found.

Third, in fact, the small volume of serum and RBCs required for the gel test make its use very attractive and suitable in pediatric testing[20]. In addition, it is of extremely valuable in antibody identification; the test procedure that consumes the most serum.

Fourth, serum or plasma can be used by the gel test. Generally, the authors restrict testing to only serum using the conventional tube test. Using plasma can be an advantage for patients with high heparin levels such as end stage renal disease patients.

In summary, through the twelve years experience using the gel test for most applications in blood group serology, it is useful in many situations. At present, there are more than 150 blood bank laboratories in Thailand using this system. Its method is easier, faster, safer and more practical than other tests. Moreover, it improves test reliability by minimizing variations between technologists stems from several features of the technique. Therefore, the misinterpretation in red blood cell serology testing can be reduced providing safer transfusion practices.

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ประสบการณ์การใช้ gel test ในประเทศไทย

อ้อยทิพย์ ณ ถลาง

ในประเทศไทยได้มีการนำการทดสอบ gel test มาใช้ในงานตรวจทางธนาคารเลือดนานถึง 12 ปี และปัจจุบันได้มีห้องปฏิบัติการที่ใช้การทดสอบนี้ในงานตรวจประจำอย่างกว้างขวาง โดยเฉพาะอย่างยิ่งในการตรวจกรองแอนติบอดี การทดสอบความเข้ากันได้ของเลือด การตรวจ direct antiglobulin test และการตรวจหาแอนติเจนของหมู่เลือดระบบต่าง ๆ ซึ่งพบว่าการทดสอบด้วยวิธีนี้มี sensitivity ดีกว่าวิธีการตรวจด้วยหลอดทดลอง นอกจากนี้การทดสอบทำได้ง่ายและสะดวก เหมาะสำหรับการตรวจกรณีที่มีตัวอย่างตรวจจำนวนมากหรือในกรณีเร่งด่วน