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The progress in pre–transfusion test technique research

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ABSTRACT

Pre–transfusion tests include ABO/RhD blood typing, irregular antibody screening and cross–matching test, which are pivotal for safe and effective clinical transfusion. The best pre–transfusion test technology should be selected to ensure the safety of the clinical transfusion, use blood resources effectively, improve the transfusion´s effect, and achieve a precision transfusion. Here we reviewed the progress of main techniques and applications of pre–transfusion tests in recent years, aiming to give clinicians a systematic understanding of current pre–transfusion test techniques in clinical transfusion treatment.

Keywords: pre–transfusion test, ABO/RhD blood typing, irregular antibody screening test, cross–matching test

Transfusion is a kind of treatment that refers to the blood or blood components administered to the patient through intravenous infusion. As an irreplaceable treatment method, transfusion is widely used in clinical departments around the world and is an important part of modern medicine.

Blood type is a genetic polymorphism in various components of the blood. Blood type incompatibility is the main cause of transfusion reaction and hemolytic disease in newborns. As a preventative measure, pre–transfusion tests, which relate to the safety and effectiveness of clinical transfusion, are designed to avoid transfusion reactions in clinic. These include ABO/RhD blood typing, irregular antibody screening and cross–matching test. Here we reviewed the progress of main techniques and applications of pre–transfusion tests in recent years, aiming to give clinicians a systematic understanding of current pre–transfusion test techniques in clinical transfusion treatment.

ABO/RhD BLOOD TYPING

In clinical transfusion, ABO system and Rh system are the most significant blood group systems. Antigen A and antigen B in ABO system, and antigen D in Rh system have strong immunogenicity, which can lead to hemolytic disease of the newborns[1,2], and is associated with various diseases.

According to the A–antigen and B–antigen on the surface of the red cell membrane, blood types are divided into type A, type B, type AB and type O. The red blood cell (RBC) agglutination test is used to identify the ABO blood type by positive and negative typing. Positive typing refers to the use of a known anti–A and anti–B blood group reagents to determine the presence of corresponding A–antigen and/or B–antigen on RBCs. Negative typing refers to the use of a known type A and type B RBC to measure anti–A and/or anti–B in serum or plasma. Currently, blood typing methods include blood type serology, gene detection, colloidal gold immunochromatographic assay and flow cytometry. Blood type serologic methods commonly used include cardboard method, micro–plate method and micro–column gel method. Gene detection technique utilizes polymerase chain reaction(PCR) and DNA sequencing.

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Blood type serologic technique

The cardboard method

In most community collection units, the blood type of voluntary blood donors is usually determined by acquiring fingertip blood and applying the cardboard method to do positive typing, which might give false blood typing. Another kind of cardboard method is by using anti−A, anti−B, and anti−D monoclonal antibodies packaged on specific cards. Wu *et al*.[3] developed a rapid identification card for the identification of ABO/Rh(D) blood type. The accuracy rate was 99.8%, as the protein may interfere with rouleaux pseudo−agglutination. Guan *et al*.[4] investigated the stability of the primary blood typing antibodies (anti−A, anti−B and anti−D IgM). Their work showed that the use of additives and freeze−drying are effective approaches to retain the activities of IgM blood group antibodies. These approaches would be further explored for the large scale development of a new generation of clinical and home−care blood testing devices.

The micro−plate method

The micro−plate method uses 96−well plate and automatic sampling to detect many samples at the same time, which is widely used in blood banks in China[5]. Hou *et al*. applied the micro−plate method to test the antibody titer of healthy donors, the titer of anti−B was lower than that of test tube method, so the test tube method needed to be verified if necessary[6]. Liu *et al*.[7] used three kinds of automatic micro−plate method to detect blood type antibodies and compared the results, they got the conclusion that the micro−plate polybrene method was rapid, sensitive and cost−effective. It was fully compatible with other test items in the working mode, and was consequently recommended to blood banks.

The micro−column gel method

The micro−column gel method is a technique to distinguish free RBC from agglutinated blood by using the molecular sieve effect of gel, which is a porous network structure macromolecule compound. This method has high specificity and sensitivity and has been widely used in clinical transfusion as a routine application of ABO/Rh(D) blood typing at home and abroad. In a study by Yu *et al*.[8], blood types were identified by using different techniques, including the micro−column gel test, immune inhibition test, absorption and elution tests. Zhong *et al*. studied the application effect of the manual micro−column gel method in the identification of ABO/RhD blood type antigens, and the clinical value was significant[9]. Yang *et al*. presented a case of ABO genotype−phenotype discrepancy resolved through a careful review of sequence chromatograms, using micro−column agglutination and direct sequencing analysis to reveal the heterozygous deletion[10].

In recent years, fully automatic blood type apparatus has become widely used in blood banks and hospitals and has improved both detection quality and efficiency[11−13]. However, it has been known to leave sub−type[14] or weak serum reactions undetected[15]. This method can be automated and use software to analyze, check and save the results, as a consequence, reduced human errors. For these reasons, this is the most widely used routine blood type test currently.

Gene detection technique

The human ABO gene is located on chromosome 9q34.2, whose gene product is a glycosyl transferase that controls the biosynthesis of ABO blood type antigens. The Rh gene is located on chromosome 1q36.11, consisting of closely linked RhD and RhCE genes in tandem. The gene detection technique is the product of modern molecular biological technology development, mainly using PCR and DNA sequencing. Compared with blood type serology, the gene detection technique is not affected by disease states and is valuable in complicated blood types[16] and sub−types[17−19], and can accurately confirm the type, so as to ensure the safety of transfusion. Papasavva *et al*. used real−time multiplex−PCR to amplify regions of the RhD gene to estimate RhD phenotype frequencies[20]. Lang *et al*. described a cost−effective method for high−throughput genotyping of the ABO system by next generation sequencing[21]. Hu *et al*. and Wu *et al*. identified ABO blood sub−types by means of serological methods and genotyping kits[16,18]. Liu *et al*. used genotyping kits and micro−column gel method to detect ABO phenotypes and genotypes in renal transplant recipients[22]. The combination of different methods helps to accurately determine the patient´s blood type. Cetinkaya *et al*. reported a donor with Bombay phenotype, which was confirmed by serological tests, pedigree studies and sequence analysis[23]. A study by Chang *et al*. showed that the genotyping method could be used instead of blood type serology for the identification of para−Bombay type in China[24].

For the above reasons, today molecular sequencing is the gold standard for blood type genetic testing. The serological technique has some limitations in the identification of complicated blood types, especially sub−types. The method of gene sequencing can be used to clarify the sometimes fuzzy results of serology. In the study of Yu *et al*., genotypic analysis was

determined by using PCR and clone−based sequencing[8]. These studies discussed the application of next generation sequencing for ABO blood type genotyping[25−28].

The gene detection technique has high sensitivity, but needs the sample DNA to be extracted first, and then there is the PCR amplification reaction, which takes time. So the detection efficiency is not high.

Colloidal gold immunochromatographic assay

In the colloidal gold immunochromatographic assay technique, particles labeled with anti−A and anti−B standard antibodies are fixed onto a nitrocellulose membrane, and antigen is conjugated with a labeled antibody to make colloidal gold particle aggregates and present a color reaction. In a study by Hu *et al*., colloidal gold was used as the tracer particle, and the colloidal gold immunochromatography assay was applied to the blood type identification field[29]. They found the use of colloidal gold immunochromatographic assay technique to have a strong practicability value, and it has since been adopted by a number of clinics .

Flow cytometry

Flow cytometry uses FITC labeled anti−A and anti−B standard antibodies, incubated and combined with RBC. The blood type is obtained by analyzing the percentage of antibodies with fluorescein combined with RBC by using flow cytometry detection software. The studies by Annika and Sun *et al*. showed that this technique has obvious advantages in identification of blood sub−types[30,31]. Due to the precision instruments and reagent´s expense, it can not be used in routine blood typing tests at present.

IRREGULAR ANTIBODY SCREENING TEST

Irregular antibodies are generally used to refer to RBC antibodies other than anti−A and anti−B. In clinic, irregular antibodies in serum are the main causes of identification of complicated blood types, difficult matching, hemolytic disease of the newborn, and adverse transfusion reaction. Before transfusion, almost all clinical transfusion laboratories will conduct an irregular antibody screening test, which is beneficial to prevent or reduce the occurrence of transfusion reaction and ensure the safety of clinical transfusion.

Currently, irregular antibody screening and identification tests are used to screen irregular antibodies in samples with known antigen type O RBC reagents or screening cells. If irregular antibodies are screened, the specificity of irregular antibodies can be identified by special RBC reagents, so the requirements for RBC screening reagents are higher. The methods used include saline test, anti−human globulin (AHG) test, enzyme test, polybrene test and micro−column gel method. Enko *et al*. determined the low−ionic strength saline−indirect antiglobulin test with an enzyme−pretreated (papain) cell panel to detect RBC alloantibodies in routine antibody screening and the identification of antibodies in patients hospitalized in Austria[32]. The results demonstrated that a high number of unwanted positive reactions with the enzyme technique overshadow the detection of "enzyme−only" RBC alloantibodies. In a study by Lin *et al*., phenotyping and genotyping were conducted on a case with antibodies against highly prevalent antigens[33]. Antibody identification was carried out using Bromelin and DTT to treat RBC. The study concluded that RBC genotyping is a potential and powerful tool for the identification of antibodies against highly prevalent antigens. Irving *et al*. evaluated the enzyme indirect AHG test and polyethylene glycol indirect antiglobulin test(IAT) to distinguish anti−D in plasma in comparison with a tube variation of the standard low ionic strength solution−IAT[34].

Many irregular antibodies have been identified using different methods. The micro−column gel method with high sensitivity is the most widely used. Yousuf *et al*. used the micro−column gel method, enzyme test and panel cells to detect anti−JK3[35]. Padmore *et al*. used the AHG test to detect anti−Kpa alloantibody in a patient[36]. Irani *et al*. reported a case of an acute hemolytic transfusion reaction due to a hemolytic anti−Le^b detected by the micro−column gel method[37]. Yang *et al*. detected the presence of IgG form anti−Mia using polybrene test and AHG method[38]. Datta *et al*. discussed a case of clinically significant naturally occurring anti−M antibody which had both IgM and IgG components in a three−year old child[39], where the blood grouping, antibody screening and compatibility testing were performed by micro−column gel method. In a study by Saini *et al*., the anti−C^w antibody of IgM type was detected using the micro−column gel method and commercial pooled cells[40]. Since most irregular antibodies are of the IgG type, micro−column gel anti−human globulin method is simple, fast and effective in detecting irregular antibodies.

THE CROSS−MATCHING TEST

The cross−matching test is an essential test to ensure the patient´s safety during transfusion, and is an important basis for determining transfusion. Through the cross−matching test, it can be ascertained that the blood between the recipient (patient) and donor (blood

0donor) has no incompatible antigens and antibodies, so as to achieve safe and effective transfusion. The methods include saline medium test, enzyme test, po−lybrene test, anti−human globulin test, micro−column gel method, electron cross−matching test.

The micro−column gel anti−human globulin method has high sensitivity and specificity and is mainly used in the cross−matching test. The automated blood bank systems used in combination with the micro−column gel anti−human globulin method can process the cross−matching test and use corresponding software to analyze and save results. Dinardo *et al*. evaluated the performance of a low−ionic−strengthsaline(LISS)−albumin enhancer to intensify antigen−antibody reac−tion and compared this performance with that of other enhancers, gel, and conventional tube testing and the impact of this method´s implementation in the C:T ratio (cross−matched to transfused RBC units) of a transfusion laboratory[41]. Garg *et al*.[42] compared the efficacy of three cross−match techniques: conven−tional tube test (CTT), tube LISS indirect AHG test, and micro−column gel method used in the blood bank serology laboratory. They found different methods of cross−matching tests could be used to improve ac−curacy of test results, and to ensure safety of clinical transfusion.

On the basis of the identification of ABO/RhD blood types and the screening of irregular serum an−tibodies, the electronic cross−matching test directly determines the blood type via computer system. The computer evaluation is used instead of the traditional serological method for the cross−matching test. In a study by Yazer *et al*., the electronic cross−matching test was found to be the most common technique, and most respondents performed the cross−matching at the time that an order for RBCs was received in the trans−fusion service (even if an order to issue the RBCs was not received) [43]. Zhou *et al*. discussed that based on the results of irregular antibody screening and direct AHG tests of patients and blood donors, electronic cross−matching test is feasible and safe for clini−cal transfusion in China[44]. For the safety of clinical transfusion, the serological cross−matching test should be used as a supplement if patients can not undergo the electronic cross−matching test.

FUTURE WORK

In recent years, clinical hospitals mostly use the micro−column gel method to do ABO/RhD blood typing, irregular antibody screening and cross−match−ing testing. The operation is not difficult, while the key is how to deal with special cases or complicated blood types correctly. The serologic blood typing is mainly limited by antibody reagents. At present, there are not many antibody reagents registered in Chinese market, and antibody reagents for rare blood types are especially uncommon. PCR and its deriva−tives are highly sensitive with a high−throughput and are easy to automate, and will be a reliable detection technique in the future. The molecular sequencing technique has unique advantages for unknown new variant genes. At present, the more precise biolayer interferometry and microscale thermophoresis can also be used for the detection of certain specific anti−gens and antibodies and for the assessment of nuclear protein−binding affinity, which have wide application prospects[45,46]. In 2016, Popescu published a trial on a plasmonic biosensor based on birefringent solid−core microstructured optical fiber applied to the detection of human blood groups[47]. Later Then *et al*. tested surface plasmon resonance (SPR) for the detection of weaker antibody−antigen interactions using the Duffy blood groups, Fy^a and Fy^b as a model[48]. In a study by Peungthum *et al*., SPR was able to indicate dif−ferent ABH antigen densities on the RBCs and nearly the same amounts of ABH substances in the saliva of strong and weak subgroups[49]. However, further development is required for SPR to become a robust clinical blood typing technique.

With improvements in the understanding of the various components and functions of blood, transfu−sion is being constantly improved by innovation. This will ensure the effective use of blood resources, im−prove transfusion effects and achieve timely and safe transfusions.

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