Original Article

Comparison Between Conventional and Automated Techniques for Blood Grouping and Crossmatching: Experience from a Tertiary Care Centre

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ABSTRACT

Context: The routine immunohematological tests can be performed by automated as well as manual techniques. These techniques have advantages and disadvantages inherent to them.

Aims: The present study aims to compare the results of manual and automated techniques for blood grouping and crossmatching so as to validate the automated system effectively.

Materials and Methods: A total of 1000 samples were subjected to blood grouping by the conventional tube technique (CTT) and the automated microplate LYRA system on Techno TwinStation. A total of 269 samples (multitransfused patients and multigravida females) were compared for 927 crossmatches by the CTT in indirect antiglobulin phase against the column agglutination technique (CAT) performed on Techno TwinStation.

Results: For blood grouping, the study showed a concordance in results for 942/1000 samples (94.2%), discordance for 4/1000 (0.4%) samples and uninterpretable result for 54/1000 samples (5.4%). On resolution, the uninterpretable results reduced to 49/1000 samples (4.9%) with 951/1000 samples (95.1%) showing concordant results. For crossmatching, the automated CAT showed concordant results in 887/927 (95.6%) and discordant results in 3/927 (0.32%) crossmatches as compared to the CTT. Total 37/927 (3.9%) crossmatches were not interpretable by the automated technique.

Conclusions: The automated system shows a high concordance of results with CTT and hence can be brought into routine use. However, the high proportion of uninterpretable results emphasizes on the fact that proper training and standardization are needed prior to its use.

Key words: Automated technique, conventional tube technique, immunohematology, pretransfusion compatibility

INTRODUCTION

Pretransfusion compatibility testing is a critical element of the entire transfusion process to enhance vein-to-vein safety.[1] Pretransfusion testing is performed in order to prevent transfusion of incompatible donor red cells that might result in an immune mediated hemolytic transfusion reaction.[2] The ABO and Rh blood grouping of the recipients and the donors, and the crossmatching between the donor and the recipient in indirect antiglobulin (IAT) phase to detect clinically significant antibodies are integral steps of the compatibility testing.[2,3] The most commonly employed technique for performing the grouping and crossmatching is the conventional tube technique (CTT). Though still considered a gold

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standard, this technique has inherent limitations in the form of elution of low affinity antibodies during washing, variability in the results due to variations in the cell-serum ratio, and lack of consistency in reporting the results due to inter-observer variability.[4,5] These assays are labor intensive, not amenable to automation, and the results are dependent on the operator.[6] The introduction of newer techniques such as column agglutination technique (CAT), solid phase red cell adherence assay (SPRCA), and erythrocyte-magnetized technique (EMT) have tried to overcome these shortcomings and bring about an improvement in the quality of testing and the reproducibility of results.[4] CAT has been shown to be more sensitive than CTT for blood grouping and crossmatching.[7] The new technologies are amenable to automation.[5,8]

Automation in blood bank serology was introduced in the developed countries in the 1960s. The automated systems are semiautomated or fully automated and are based on either of CAT, SPRCA, and EMT. Automation in blood banks is being adopted by more and more centers and is rapidly becoming a standard testing technology in developed nations.[9] Automation provides the advantage of improving the quality of testing by decreasing human errors in sample identification, thus reducing the risk of transfusion reactions due to mismatched blood transfusion.[10,11] Documentation, traceability and archiving of results are other major advantages of automation.[4] In India, automation has come up in a big way with the larger centers shifting to totally automated platforms for serologic testing.[4] Previous studies have evaluated the performance of fully automated systems and found them equally sensitive and reliable as the CTT.[12,13]

The present pilot study was undertaken at a tertiary care center in India to evaluate the performance of fully automated immunohematological system and to assess its role in pretransfusion compatibility testing in the Indian scenario.

The primary objective of this study was to compare the efficiency of the automated system and the CTT for pretransfusion testing. The study also aimed at identifying the areas responsible for maximum errors in automated techniques and to provide a possible solution to minimize the same.

MATERIALS AND METHODS

This prospective study was performed at the Department of Transfusion Medicine of a tertiary care center in India. The annual workload of the center includes more than 30,000 donor unit collection, and approximately 40,000 crossmatches. The study was conducted from January 2011 to September 2012. Ethical approval was granted by the Institutional Ethics Committee as per letter no. EC/80/2011.

The pretransfusion blood grouping and crossmatching tests were performed manually by CTT and the same samples were subjected to the automated platform of Techno TwinStation.

Techno TwinStation

Techno TwinStation (Bio-Rad Laboratories, USA) is a fully automated analyzer for immunohematology testing in the blood bank. Blood grouping is performed by microplate method on LYRA component of the system. Crossmatching, antibody screening and identification and rare antigen typing are performed by CAT using sephadex gel.

Sample selection

Blood grouping

As a pilot study, a total of 1000 blood samples (650 blood donors and 350 recipient patients) were selected for comparison of ABO and Rh blood grouping by the CTT and by the automated microplate LYRA system. The samples of the donors were collected at the site of blood donation. The samples of the patients that were collected in clinical wards and sent to the blood bank for blood grouping were included in this study. All the samples were collected in BD Vacutainer® Tubes with K2EDTA and tested on the same day of collection. Samples adequate in the quantity required for testing by both the techniques were included in the study. Hemolyzed and/orlipemic samples were excluded from the study. Samples of neonates and infants up to 6 months of age were also excluded in view of the incomplete development of ABO antibodies in their plasma.

The samples were selected by simple random sampling by lottery method.

Crossmatching

A total of 269 samples received in the blood bank from multitransfused patients and multigravida females during the study period were compared for 927 crossmatches. This selection aimed at including the samples with antibodies so that the concordance of the two techniques and the

strength of reaction could be compared for incompatible crossmatch results. The samples were collected in BD Vacutainer® Tubes with K2EDTA. Samples adequate in the quantity required for testing by both the techniques were included in the study. Samples that were hemolyzed and/or lipemic were excluded from the study.

Methodology

Blood grouping and crossmatching were first performed on Techno TwinStation (machine). To remove the personnel bias leading to performance variation, it was ensured that blood grouping and crossmatching of one particular set of samples by both the techniques was performed by the same technologist. In order to prevent observer bias and transfer of information between the two methods, all the specimens for manual testing were aliquoted and coded.

Blood grouping

The forward and reverse grouping of 1000 samples was first performed using precoated microplate as per the manufacturer's instructions. For forward grouping, 5% cell suspension was prepared by the system and 10 μL of it was added to each of anti-A, anti-B, anti-D reagents and to the control well. For reverse grouping, 50 μL of plasma was added to each of 50 μL of A1 cells and B cells. After sampling, the microplates were transferred to LYRA component of the automated system where the microplates were centrifuged for 10 min. After 10 min of centrifugation, the pictures were captured by the camera in the machine, and the interpretation was displayed. The blood grouping of these 1000 samples was then performed by CTT and interpreted according to AABB Technical Manual.[14] Briefly, 1 drop of 2–5% red cell suspension was added to each of anti-A, anti-B, anti-A, B and anti-D monoclonal reagent (Tulip Diagnostics, India) in test tubes for forward grouping. For reverse grouping, 1 drop each of A1, B and O pooled cells was added to two drops of plasma in test tubes. The tubes were incubated and centrifuged as per the reagent manufacturer's instructions. Agglutination in forward grouping and either hemolysis or agglutination in reverse grouping were interpreted as a positive reaction which was graded. The ABO and Rh type of samples were recorded from the results of forward and reverse grouping. All Rh negative samples were tested further for weak D antigen using antiglobulin reagent (Tulip Diagnostics, India). In case of a discrepancy between forward and reverse grouping, additional reagents anti-A1 and anti-H were used along with other guidelines for resolving the discrepancy.[14]

The test results of automated and manual technique were compared. The results were classified as concordant, discordant and uninterpretable.

To evaluate the time taken by each technique for blood grouping, 3 batches consisting of 1, 18 and 36 samples were run by both the techniques. Each batch was run 20 times and the average time taken for each batch was calculated.

Crossmatching

A major IAT crossmatch was performed using patients' plasma and donor red cells using CAT microtubes (gel card) with polyspecific antihuman globulin (AHG) gel cards (Bio-Rad Laboratories, USA) on the machine as per the manufacturer's instructions. Donor red cell suspension was prepared by the machine by adding 10 μL of cells to 1 ml of low ionic strength saline (LISS) solution to give a final concentration of 1%. The system pipetted 50 μL of suspension to a microtube. To this, 25 μL of patient's plasma was added. After incubation at 37°C for 15 min, the cards were centrifuged at 1000 rpm for 10 min and the results were read. A total of 927 crossmatches were performed on 269 patient samples.

Major IAT crossmatch on the same samples was performed using polyspecific antiglobulin reagent by CTT.[14] Manufacturer's instructions were followed for using the antiglobulin reagent (Tulip Diagnostics, India). Briefly, 1 drop of 3–5% suspension of donor red cells was mixed with 2 drops of recipient plasma. The mixture was incubated for 60 min at 37°C, and washed 3 times with 0.9% saline. One drop of antiglobulin reagent was added followed by centrifugation at 1000 rpm for 1 min. The results were interpreted as positive or negative. The strength of positive (incompatible) reactions was recorded.

The results of crossmatches by the automated system and CTT were compared. The strength of reaction was graded from 1 to 4 in case of the positive concordant samples.

The samples showing discordant results in a cross match in the IAT phase were subjected to antibody screening and identification by manual CAT using 3-cell and 11-cell panels (Bio-Rad Laboratories, USA) as per the manufacturer's instructions.

Statistical tests

The percentage of concordant, discordant and uninterpretable results was calculated for grouping and crossmatching. The results were analyzed by applying

appropriate statistical tests using SPSS software version 16 (Biorad Laboratories USA). Unpaired *t*-test was used to analyse the time taken by CTT and the automated system for blood grouping. Mann–Whitney test was used to compare the strength of agglutination reaction by the two techniques. $P < 0.05$ were considered significant.

RESULTS

ABO and Rh blood grouping was performed on 1000 samples tested in parallel by the CTT as our standard procedure and the microplate LYRA system as a part of Techno TwinStation. The results and time taken by both the techniques were compared. A total of 927 crossmatches were performed on 269 samples from multitransfused patients and multigravida females. The results of crossmatches and strength of positive reactions by CTT and by CAT on the machine were compared.

The two techniques showed concordance of results for 942/1000 (94.2%) samples tested for blood grouping. There was discordance for 4 out of 1000 (0.4%) samples while 54/1000 (5.4%) samples were uninterpretable on the automated system initially. The result was termed "uninterpretable" when displayed so by the machine. These 54/1000 samples did not show any difficulty in interpretation of blood groups when performed by CTT.

The four discordant results were due to the inability of the microplate system to detect weak D, hence giving Rh (D) negative results by the machine. As per the protocol described in "methods," these Rh (D) negative samples were subjected to a test with specialized reagent using CAT on the machine. This led to detection of weak D antigen by the automated system. After this discrepancy was resolved, the results became concordant in 946 (initial 942 + 4) samples.

Out of the 54 uninterpretable results, 5 (9.26%) results were due to blood group variants. They included A2 with anti-A1 in serum (three samples) and Bombay phenotype (two samples). These were not interpreted by the LYRA system due to the lack of specialized anti-A1 and anti-H antisera gel cards as they are not a part of routine blood group testing. These five samples were subjected to repeat the test on the automated system using the specialized anti-A1 and anti-H gel cards. The results similar to those given by CTT were obtained. Thus, a total of 951/1000 samples (95.1%) gave concordant results with both the techniques while 49/1000 samples (4.9%) remained uninterpretable by the machine (reasons listed in Table 1). These samples had to be resolved manually. The sensitivity, specificity, positive predictive value and negative predictive value could not be calculated as the automated system did not give false positive or false negative results in the blood grouping test. The uninterpretable results were encountered more frequently in the initial part of the study (36/49 in first 6 months), their number reduced over time (13/49 in next 6 months). No uninterpretable results or errors were observed in manual CTT.

The time taken by the two methods for blood grouping was compared for batches with different number of samples. Unpaired *t*-test was applied, which showed that the machine took significantly longer time ($P < 0.001$) when only one sample was processed. However, the machine took significantly shorter time ($P < 0.001$) when the batches of 18 and 36 samples were compared [Table 2].

A total of 927 crossmatches were performed on 269 samples of multitransfused patients and multigravida females. The cross-matching was performed in IAT phase by both CTT and the automated gel technique. The results of the two techniques were compared. The two techniques showed concordance of results in 887/927 crossmatches (95.6%). Concordance means the crossmatch results were either compatible (832/887 concordant results) or incompatible (55/887 concordant results) in the IAT phase using both the techniques.

Discordant results between the two techniques were observed in 3/927 crossmatches (0.32%) performed over two samples. They were incompatible with gel technique in the IAT phase but compatible with tube technique in the IAT phase. Further antibody identification of these two samples using 3-cell antibody screening and 11-cell antibody identification panel revealed anti-M and anti-K antibodies respectively.

Of the 927 crossmatches by the gel technique on Techno TwinStation, 37 (3.99%) results were not interpretable and had to be resolved manually. These results were due to interference by fibrin in 18/37 cases (48.64%), improper samples of the donors with clots in 12/37 cases (32.43%), insufficient quantity of donor

Table 1: The number of samples with uninterpretable results on automated system due to different causes

Sample errors	Operation errors	Automation interpretation errors	Errors due to cold agglutinin	Total uninterpretable samples
31	09	08	01	49

Table 2: Average time taken by each technique for blood grouping

Number of samples in one batch (× number of runs per batch)	Manual tube technique (time in minutes)	Automated system (time in minutes)	*P*	df	95% CI
1 (×20)	12.17 (SD=1.60)	20.5 (SD1=2.57)	<0.001	38	−9.70 - −6.96
18 (×20)	40 (SD=1.90)	35 (SD2=1.47)	<0.001	38	3.91-6.09
36 (×20)	65.8 (SD=2.13)	56.17 (SD3=2.17)	<0.001	38	8.25-11.0

SD: Standard deviation, df: Degree of freedom, CI: Confidence interval

samples in 7/37 cases (18.91%). The sensitivity, specificity, positive predictive value and negative predictive value could not be calculated as the automated system did not give false positive or false negative results in view of the uninterpretable results. The uninterpretable results were obtained more frequently in the initial part of the study (29/37 in the first 6 months); their number reduced over time (8/37 in the next 6 months). No uninterpretable results were observed with the manual CTT.

Mann–Whitney test was applied to compare the difference in strength of reactions of 55 incompatible crossmatches between the CTT and the CAT on the machine. The mean ranks were compared which showed a *P* value of 0.014 (<0.05) indicating higher sensitivity of CAT compared to CTT.

DISCUSSION

The conventional manual tube technique is still considered as a gold standard for pretransfusion compatibility testing. However, there is a need to minimize the disadvantages associated with manual tube technique. Hence this pilot study was undertaken in India with the primary objective of comparing the efficiency of automated technique with CTT for immunohematological testing.

The high concordance of results in our study (95.1% for blood grouping and 95.7% for crossmatching) indicates that Techno TwinStation system can perform blood grouping and crossmatching with accuracy that is comparable to manual techniques. These results are similar to other studies comparing the automated gel system with CTT technique.[12,15]

The discordant results in our study were due to Rh typing similar to the previous reports.[13] The microplate technique uses monoclonal anti-D reagent which does not detect weak Rh (D). The automated system has in place a separate test wherein the Rh negative samples can be subjected to specialized weak D testing, eliminating such errors. The errors due to blood group variants occurred because anti-A1 and anti-H are not used as a part of the routine microplate testing; however the reaction pattern with the standard reagents by LYRA was the same as that of the standard tube technique. In other studies validating automated systems, the most common identifiable cause of undetermined ABO and Rh typing was weak subgroups of ABO system.[16] When these samples showing blood group variants were subjected to further testing using required reagents, the discrepancy could be resolved, and results were comparable to standard tube technique. However, the specialized reagents (anti-A1 and anti-H gel cards) are expensive and lead to additional cost per test.

Uninterpretable results were observed in 49/1000 samples processed on the automated system. In previous studies, the proportion of undetermined results for automated and semiautomated systems ranged between 2.5% and 13%.[16] These results reflect the problems encountered in standardizing the collection into vacutainers and nonadherence to the manufacturer's instructions on a few occasions. Another study has reported a 3% and 5.8% sample related problems in donor and patient population respectively leading to uninterpretable results during the evaluation of an automated system for ABO and Rh grouping.[13] In our study, sample related problems occurred in 4.15% (27/650) of donor samples and 1.14% (4/350) of patient samples. The higher percentage of problems in donor samples was the result of lack of familiarity regarding the use of vacutainers for sample collection, inadequate mixing and inadequate quantity of sample collected. The collection of patient samples in vacutainers has been an established practice in our hospital and hence a lower rate of problems was observed with patient samples. The errors related to sample collection were in the initial part of the study and reduced with regular use and training.

Only one patient sample (0.1%) showed the presence of cold agglutinin in our study which was resolved by the standard tube technique. Inclusion of samples from different groups of patients with numerous disease states might be expected to cause interpretation problems with any automated system.[17]

In our study, the machine took longer time as compared to CTT when only one sample was processed for blood grouping. However, the time taken to process larger batches of 18 and 36 samples was significantly

lower with the automated system. A study has shown that the CTT for blood grouping is the fastest but is not suited for batch testing, whereas the automated technique is more suited for blood grouping in terms of batch testing.[4] The main advantage of the automated system in ABO grouping is the increased "hands-off time" and traceability due to the generation of records with photographs at the end of the procedure. The significant amount of hands-off time can be utilized by the technologist for other laboratory procedures, especially in centers with a large workload.

Of the 927 crossmatches performed by the manual and the gel technique, uninterpretable results were obtained in 37/927 crossmatches (3.99%) due to sample related problems similar to other previous studies.[13,16] These uninterpretable results clearly show problems associated with sample collection in blood donors which were due to lack of familiarity with vacutainer collection. These problems reduced in number with continued use likely because of the training and experience of the technical staff.

Three crossmatches involving two samples were incompatible only by the automated gel technique and had anti-K and anti-M but were not detected by manual IAT leading to discordant results. Also, CAT on the machine gave the higher strength of reactions as compared to CTT. These results are similar to those in other studies which concluded that the gel test is at least as sensitive as CTT using LISS, with a better balance of sensitivity and specificity which is in agreement with our study.[18,19]

The cost per test was found to be three times higher with the automated system as compared to CTT for blood grouping. The cost per test of IAT crossmatching was found to be 40% higher with the automated system as compared to CTT. However, this cost does not include the expenditure associated with the employment of additional manpower required for manual testing by CTT. As per the previous reports, the automation in immunohematology is expensive and usually requires a large initial investment. The cost per test eventually decreases as the number of samples processed increases.[4] Hence, the decision to use the automated system will largely depend on the workload of the blood bank as well as the available financial and manpower resources.

Limitation of the study

This study is a baseline pilot work with the limitation of time and resources. An analysis of the automated system after full implementation for routine use with regular audits will provide deeper insight into a broader perspective.

CONCLUSION

The high level of accuracy with a shorter turnaround time for blood grouping and high sensitivity for crossmatching indicate that the automated systems can be incorporated into routine use in blood banks with a large workload. However, the high proportion of uninterpretable results emphasizes that training and standardization are required prior to its use. The competence of trained technologist is required to validate the results of the automated system.

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Conflicts of interest

There are no conflicts of interest.

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