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COMPARATIVE STUDY OF BLOOD GROUPING USING CONVENTIONAL TUBE AND GEL METHOD- EXPERIENCE FROM A TERTIARY CARE CENTRE (SIR T HOSPITAL, BHAVNAGAR).

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ABSTRACT

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

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

Materials and Methods: A total of 1000 samples were collected and blood grouping done by the conventional tube technique (CTT) and the Gel Card Method.

Result: Blood group testing was performed on 1000 blood samples by conventional test tube method and Matrix Gel Method. The results were analysed.

onclusion: The gel method found to be a rapid and more reliable procedure than CTT and can be used in place of conventional method.

KEYWORDS : Blood group testing; Conventional tube, Matrix gel card.

Introduction

Red blood cells are differentiated from each other by their surface antigen structures. Karl Landsteiner first discovered[1] the ABO blood group (BG) system in 1900 and rhesus (Rh) BG later [2], to which safe blood transfusion is greatly attributed. Spin tube method is the conventional method for blood grouping and cross matching in transfusion medicine. This technique is considered gold standard, but it 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[3],[4]. Automatic technique has 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[14],[15]. Lapierre who introduced gel tests[5] using principle of controlled centrifugation of red cells through sephadex gel contained within a microtube. Blood serum or cells are mixed with anti-A, anti-B, and anti-D reagents in microtubes with controlled incubation and centrifugation. The gel particles trap the agglutinates and non-agglutinated blood cells pass through the column.

The present study was carried out to evaluate the efficacy of gel card method and to compare with the conventional tube method for Blood grouping.

Material and Method:

This prospective study was performed at the Blood bank of a tertiary care center in sir T hospital, Bhavnagar. A total of 1000 blood samples (from healthy blood donors) were selected for comparison of ABO and Rh blood grouping by the CTT and by the Matrix Gel card method. Blood grouping of one set of samples by both the techniques was performed by the same technologist to remove the personnel bias.

Hemolysed and/or lipemic samples were excluded.

For Conventional tube method, both forward and reverse grouping done using anti-A, anti-B, anti-AB and anti-D monoclonal reagent (Tulip Diagnostics, India) and blood bank prepared pooled cells. The ABO and Rh type of samples were recorded from the results and all Rh negative samples were tested further for weak D antigen using antiglobulin reagent (Tulip Diagnostics, India). For Matrix gel card method, prepare 5% red cell suspension for forward grouping and prepare a 0.8% red blood cell suspension for reverse grouping for blood grouping using Matrix diluent-2 LISS. Label the Matrix Octoplus Complete Grouping Card with donor's name. Remove the aluminium foil by pulling it backwards. Pipette 10 microlitre of 5% donor's red cell suspension to microtube 1 to 5 , 50 microlitre of 0.8% known A1 cell suspension , known B and known O cell suspension to microtube 6,7 and 8 respectively and then 50 microlitre of donor's plasma or serum to microtube 6,7 and 8. Incubate card at room temperature and centrifuge for 10 minutes. All samples are given unique BBR No. and results are recorded.

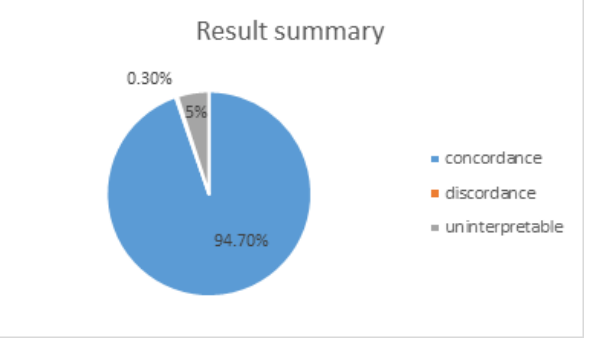
Positive reaction recorded as agglutination at the top or dispersed and Negative reaction if red cells settle at the bottom forming a compact button. The reaction strength may be recorded from +4 to negative and mixed field agglutination if RBCs form a line at the top and red blood cells form a compact button at the bottom.

The control microtube must be negative to validate the forward grouping results.

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, 3 batches consisting of 1, 18 and 36 samples were run by both the techniques.

Results

The two techniques showed concordance of results for 947/1000 (94.7%) samples tested for blood grouping. There was discordance for 3 out of 1000 (0.3%) samples while 50/1000 (5%) samples were uninterpretable on the automated system initially. These 50/1000 samples did not show any difficulty in interpretation of blood groups when performed by CTT.



The three discordant results were due to the inability of the gel system to detect weak D, hence giving Rh (D) negative results by the machine. That was solved by conventional tube method for weak D antigen.

Out of the 50 uninterpretable results, 5 (10%) results were due to blood group variants. They included A2 with anti-A1 in serum (five samples). These were not interpreted by the Matrix Gel card method due to the lack of specialized anti-A1 antisera gel cards. These samples had to be resolved manually. The uninterpretable results were encountered more frequently in the initial part of the study (33/45 in first 6 months), their number reduced over time (12/45 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.

Discussion

There is a need to minimize the disadvantages associated with manual tube technique. Hence we have undertaken this study with the primary objective of comparing the efficiency of Gel card method with CTT for blood group testing.

The high concordance of results in our study (94.7% for blood grouping) indicates that Matrix Gel card method can perform blood grouping with accuracy that is comparable to manual techniques. These results are similar to study done by Shin SY et al having concordance ranging from 99.6% to 100%.[6] Results are also comparable to study done by Schoenfeld H et al having concordance of 94.2%.[7] and concordance of 98.8% in study done by Sandler SG at al.[9]. Another study showed 98% concordance in forward and RhD grouping, but weaker reactions in reverse grouping by Takako Ono1 et al.[11]

The discordant results(0.3%) in our study were due to Rh typing are similar to other study done by Swarupa Nikhil Bhagwat at al, giving 0.4% discordance.[10] The discordance of The discordant results in Gel technique are due to inability to detect weak Rh (D).In study done by Langston MM at al , weak D was detected in all 10 specimens tested by both tube method and Gel method[12].The errors due to blood group variants usually occurs because anti-A1 and anti-H are not used as a part of the routine testing; In other studies validating automated systems, the most common identifiable cause of undetermined ABO and Rh typing was weak subgroups of ABO system.[8] These samples showing blood group variants can be subjected to further testing using required reagents, the discrepancy can be resolved, and results may be 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.

Red cell mediated discrepancies may be due to loss of antigens on cells, acquired B-antigen like activity, antibody coated on red cells and Serum mediated discrepancies are due to unexpected antibodies, rouleaux formation, loss of antibodies and hemolysis were not observed as we have taken healthy adult donors in our study. But inclusion of samples from different groups of patients with numerous disease states can cause interpretation problems with any automated system[13].

Uninterpretable results were observed in 45/1000(4.5%) samples processed on the automated system. In other study done by Screnock D at al ranged between 2.5% and 13%. [8] Also another study by Sandler SG at al, has shown 3% uninterpretable results by automated technique due to clots, hemolysis or lipemic samples .[9] These results reflect the problems encountered in standardizing the collection into vacutainers and non-adherence to the manufacturer's instructions on a few occasions. The errors related to sample collection were in the initial part of the study and reduced with regular use and training. The uninterpretable results initially were (36/49 in first 6 months), their number reduced over time (13/49 in next 6 months) in study done by Swarupa Nikhil Bhagwat at al, that is similar to our study [10].

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 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 [3].

The main advantage of the automated system in ABO grouping is the increased "hands-off time" that can be utilized for other laboratory procedures, especially in centres with a large workload.

The cost per test was found to be three times higher with the automated system as compared to CTT for blood grouping. However, this cost does not include the expenditure associated with the employment of additional manpower required for CTT. The automated blood grouping techniques are expensive and requires a large initial investment. The cost per test eventually decreases as the number of samples processed increases [3]. 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.

Conclusion

The high level of accuracy with a shorter turn-around time for blood grouping indicate that shifting from manual technique to automated technique should be done as a routine use in blood banks having a large workload to provide great patient care with less turn around time. But training of staff and standardization is required to prevent errors and uninterpretable results.

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