ISSN: 2581 – 3633
PubMed - National Library of Medicine - ID: 101745081

International Journal of Medical Science and Advanced Clinical Research (IJMACR)

Available Online at:www.ijmacr.com

Volume – 5, Issue – 6, November – December - 2022, Page No. : 279 - 286

Comparative Evaluation of Point of Care Erycard 2.0 Against Conventional Slide Agglutination and Gold Standard Tube Agglutination technique for ABO and RH Grouping

[1]Dr. Kahkasha Aalam, [2]Dr. Chandni Naresh Krishnani, [3]Dr. Anil Kumar Verma, [4]Dr Firoz Sheikh, [5]Dr Omika Meshram, [6]Dr Chandan Bajad

Corresponding Author: Dr. Chandni Naresh Krishnani, A 5, Capital city Phase 3, Saddu, Raipur Chhattisgarh- 492001.

How to citation this article: Dr. Kahkasha Aalam, Dr. Chandni Naresh Krishnani, Dr. Anil Kumar Verma, Dr Firoz Sheikh, Dr Omika Meshram, Dr Chandan Bajad,"Comparative Evaluation of Point of Care Erycard 2.0 Against Conventional Slide Agglutination and Gold Standard Tube Agglutination technique for ABO and RH Grouping", IJMACR-November – December - 2022, Vol – 5, Issue - 6, P. No.279– 286.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Background: ABO and RH grouping system is the most important test required in many clinical scenarios including blood donation and urgent transfusion in the emergency department and operating room, for blood grouping only few methods are available I.e,slide method, microplate technique and column agglutination technique, out of which only slide method is suitable for offsite use (camps and rural settings). Slide method has lots of limitations like storage of the reagents, drying and mixing of reaction mixtures leading to misinterpretation of results, but still this method is widely used in several blood banks in India. Hence efficient and rapid reporting in rural settings is the need of hour. This study evaluated the use of Erycard 2.0 Against conventional slide agglutination technique and tube agglutination technique forblood grouping as a point of care testing especially in rural settings.

Aim & 0bjectives: The primaryobjective of this project was to assess the effect of temperature and ease of use on ERYCARD 2.0. The secondary objective was to compare the time span and stability of result of ERYCARD 2.0 over slide agglutination technique and tube agglutination technique.

Method: Total 150 blood samples were collected in the blood bank of tertiary care hospital. The accuracy and time required for ABO and Rh blood grouping results were compared using Erycard 2.0 and conventional slide technology as well as Tube agglutination method serving as the gold standard. Erycard 2.0 as a device was also assessed for its stability under various storage circumstances and the stability of the results for up to 48

hours. A poll of paramedical workers was used to assess the ease of use of Erycard 2.0.

Result: Erycard 2.0 and the slide approach both showed 98 percent concordance with the tube method. The average time taken per test by the slide technique was 2 minutes and 6 seconds, and the time taken by Erycard 2.0 according to the user manual instructions was 5 minutes and 6 seconds, although stable results in Erycard were acquired in an average time of 2 minutes and 8 seconds. Erycard 2.0 did not deviate from the findings after keeping it under two different temperature conditions. Even after 48 hours of testing and storage at room temperature, the outcome remained unchanged. According to an ease-of-use survey, Erycard 2.0 was more acceptable to paramedics as it is user friendly, easy to learn, perform and interpret results and chances of injury are less. When compared with tube agglutination the three discrepancies were found by both slide technique and Erycard 2.0. All the descripencies were of ABO type, and no RH type discrepancy was seen. [Table 2]

Conclusion: This study showed that Erycard 2.0 may be used as a point of care device for ABO and Rh blood grouping especially in resource constrained setting like rural settings, health camps, bedside blood grouping due to its comparable results with slide testing method with many additional advantages over slide method like better accuracy, stable results, stability of device in wide temperature variation, the risk of contamination of blood sample is much less than that of the slide method in off-site use , less skill needed and easier interpretation of the results.

Keywords: Erycard, Blood grouping, ABO & Rh.

Introduction

ABO RH grouping system is the most important test required in many clinical scenarios including blood donation and urgent transfusion in the Emergency Department and operating room.[1,2] In general, more than three hundred genetically-different blood groups have been determined; however, the ABO and Rh Blood Group system has fundamental importance in transfusions due to its two characteristics : Firstly, antibodies of the ABO system are present within the serum of almost every person who doesn't have the corresponding antigen, which is not seen in other blood group system. Secondly, the all agglutinins of the ABO system fix complement and are capable of causing intra vascular hemolysis of incompatible red cells. For these reasons, an error in ABO grouping of a patient or donor could turn out to be fatal during blood transfusion process.[3,4] Even though O negative can be utilized to avoid immune response, the availability of good ABO and Rh typing technique can improve medical care and decrease utilization of emergency products, which may be short in supply.[2]

There is a wide variety of analytical tests and tools available for ABO and Rh blood group typing, ranging from old classical ones such as slide or tube tests, to relatively new method of microplate technique and column agglutination technique. Of all these four techniques, the latter three methods are only suitable for laboratory testing of ABO and RhD blood grouping of the donor because besides the test reagents, these methods require well trained medical personnel and additional equipment such as centrifuge for the testing procedure.[1,5] Slide/tile method is the only portable and simple method that is feasible and appropriate for offsite donation drive or bedside blood group confirmation in

rural settings and camps.[5] Grouping by slide method has a lot of limitations such as drying up of reaction mixture, difficulty in interpretingweaker reactions, mixing of reaction mixtures, misinterpretation due to inadequate mixing of RBC and antisera, no reproducibility, and lots of others.[1] Despite being less sensitive, it is still used as conventional and usually point-of-care (POC) technique because of its simplicity and ease of use and prompt results and is very useful in emergency cases.[1,4] Beside feasibility, simplicity and portability of the slide method, it has limitations to be used in rural settings and health camps i.e. it requires the testing reagents to be brought to offsite where it may not be kept in the optimal storage temperature of 2-6^{0}C, more so, the slide/tiles that will be used for ABO and RhD testing will be contaminated with donors blood and these will pose a risk of contamination to the operator as well as the environment.[5]

Recently, a new Point of Care device Erycard 2.0 has been launched for testing ABO and Rh blood groups which is based on the principle of lateral flow guided by capillary action.[5] In this the appropriate reagents are pre-dried at their respective sample pad beneath the sample well namely Anti-A antibodies in sample well A, Anti-B antibodies in sample well B and Anti-D antibodies in sample well D. The auto control does not contain any antibodies in sample well (Ctrl) and acts as a negative control that serves to validate the test results.[6] This is similar to the slide grouping in terms of simplicity, ease of use, no requirement of equipment or extensive training, and also overcomes several limitations of slide grouping like accuracy of result, stability of result, and maintaining storage temperature for test reagents.[1]

In view of the above, the primary objective of the study was to compare sensitivity and specificity of new point of Care Erycard 2.0 Against Conventional Slide Agglutination with gold standard Tube Agglutination technique (as this method gives incubation time)[3,7] for ABO and RH Grouping. Also to evaluate the time for testing, stability of result, stability of device in different temperature and ease of use of the Erycard 2.0

Materials and methods

ThisCross-sectional Analytical study was carried in the department of Pathology, at Raipur Institute of Medical Sciences, Raipur from 1st July to 31st August 2021. Under all aseptic precautions, samples were collected from the antecubital vein in a 2-ml disposable syringe with 24G needle from all the voluntary blood donors who donated blood at the blood bank Unit attached to the tertiary care hospital after due approval from institutional Ethics Committee (IEC). (Approval letter no. RIMS/DEAN/1036-D/ 2021 dated from IEC RIMS, Raipur Registration no. ECR/969/Int./CG/2017). Two ml of EDTA anticoagulated blood was collected from donors who were included in the study. Donors not consenting for the study were excluded.

A questionnaire was used for assessing the ease of Erycard 2.0. It had 5 questions with 4-point likert scale where 4 stands for strongly agree and 1 stands for strongly disagree. Mean score was calculated for each of the 5 questions, which came out to be more than 3 for each question. Total score was calculated as sum of scores for individual questions. Score of 1 was given for strongly disagree, 2 for disagree, 3 for agree and 4 for strongly agree. Thus total maximum score for each question was 60 and minimum score was 15.

The data was entered in to Microsoft excel and data analysis was done using SPSS V21.0. software.

Blood grouping by Erycard 2.0 method[6]: Open the pouch and Label the ERYCARD 2.0 test device with the

patient's ID and date. For finger prick samples, sample collection loop provided in the device pouch was used and for samples collected in anticoagulant, 5µl micropipette was used. Using a micropipette/sample collection loop add 5 µl of the donor's whole blood sample to each of the sample wells indicated as 'S'. When using a micropipette, ensure that only the blood drop is in contact with the pre dried reagent on the sample pad and absorbed by it. In case the micropipette tip touches the sample pad, discard the tip and use fresh tip for dispensing the sample into the subsequent sample well.

4 sample collection loops are provided with each ERYCARD 2.0 device for dispensing sample on A, B, D and Ctrl. After waiting for one minute add two drops of the reagent buffer to each of the reagent wells indicated as 'R'. Wait for 3 minutes after addition of reagent buffer to interpret the test results.

The autocontrol should show a colourless patch before the results can be confirmed. If the autocontrol pad displays a colour (invalid result) then the test results shouldn't be interpreted.

Then the blood samples were grouped with slide and tube method

For tube method Centrifugation of the EDTA blood sample at 3000 rpm for 3 min. Plasma and cell was separated. 5% of RBC cell suspension was used for cell grouping by using differentantisera's and separated serum for used for reverse blood grouping by using different A, B, O group pooledcells.

For slide method EDTA blood samples and A, B, D antisera were used and observed agglutination of RBCs macroscopically as well as microscopically.

Comparison of time span for blood grouping by Erycard 2.0 against slide method

This comparison was performed on ten blood donors. Time taken to perform grouping by Erycard 2.0 and that by slide were measured using a stopwatch starting with finger prick and ending at interpretation of result.

Assessment of stability of Erycard 2.0 due to temperature variation

To study the effect of temperature, 10 Erycard 2.0each were kept in two different setups for 15 days and then were tested simultaneously. The two setups included high temperature and low temperature. Ten Erycard 2.0,each along with thermometer were kept in 2 container and were placed in incubator for maintaining high temperature (35°C - 45°C) and refrigerator for maintaining low temperature (2°C - 8°C) respectively. A control group of 10 Erycard 2.0wasalso kept at the optimum temperature (2°C–30°C)[15], as described in the manufacturer's instructions. In both the settings, the container and thermometer were checked every day for 15 days. The cards were taken out on the 16th day. Using 10 known donor blood samples (containing both Rh D positive as well as Rh D negative samples), blood grouping was performed on devices kept in both the setting and control simultaneously. The results were recorded and compared.

Assessment of stability of result in Erycard 2.0

For checking the stability of the results obtained by Erycard 2.0, blood grouping of unknown twenty donor samples were performed. The results were recorded and be considered as 0 h. The devices were left at room temperature and interpretations were recorded at the end of every 12 h, and this is done till 48 h.

Assessment for ease of use of Erycard 2.0: To assess ease of use of Erycard 2.0 a survey were conducted for 15 paramedical staff including nursing staff and laboratory technicians working in tertiary care hospital.

The questionnaire were having five questions which was based on a 4 point Likert scale. All participants were asked to perform the test after brief explanation of the technique. After performing the test, they were asked to fill up the questionnaire individually

All data was maintained in Microsoft office Excel. All statistical analysis was carried out using Excel and Appropriate Statistical tools were applied wherever required like tests of proportion and tests of significance.

Results

Out of total 150 blood donors, incidence of different blood groups was as follows, A+ 41, B+ 50, AB+ 17, O + 29, A –Ve 7, B-Ve 5, O-Ve1 (shown in Graph 1).

Comparison of sensitivity of Erycard 2.0 over slide and tube agglutination technique

A total of 150 healthy, volunteer blood donors were tested both by Erycard 2.0 and by conventional slide method and compared with gold standard tube method. In 147/150 (98 %) of the samples, accurate results were obtained. All the three discrepancies were seen in both slide method and Erycard 2.0. The positive predictive value of Erycard and slide method was 100%, while the sensitivity of Erycard and slide method was 98% [Table 1]. All the three discrepancies were of ABO type, and no RH type discrepancy was seen. [Table 2]

Comparison of time span for testing of blood group by Erycard 2.0 over slide agglutination technique

On ten samples, the time it took to perform blood grouping using Erycard 2.0 and the slide method was recorded using a stopwatch [Table 3]. The average time taken by slide method and Erycard 2.0 is shown in graph. [Graph 2]

Assessing the stability of device due to temperature variation: Temperature variations had no effect on the accuracy of blood grouping by Erycard when the devices were stored in two different setups for 15 days each.

Assessing the stability of results in Erycard 2.0

At 12 h intervals until 48 h, all twenty devices showed no deviation from the initial observed result.

Assessing the ease of use of Erycard 2.0

The survey included fifteen members of the blood bank's paramedical staff. The mean score for each question was calculated based on the responses from the questionnaires. [Table 4]

Discussion

ABO and Rh blood grouping can be performed by various methods like conventional slide and tube methods. Other methods are gel card and advanced microplate technique and molecular methods as well. Simple Slide method can be used for offsite donation drives/ camps especially in rural settings. This study evaluated the use of Erycard versus Conventional methods including slide and tube agglutination methods.

In the present study, out of 150 donor samples, 3 samples showed discrepancies in blood grouping by Erycard versus conventional methods. Both Erycard and slide method had sensitivity 98%. The discordant results may be because of weaker antigenic expression shown by some of the blood samples or some of them may be having room temperature reacting alloantibody which may have given positive reaction with respective cells. Additional ancillary tests were required for further evaluation of this discrepancy which could not be performed due to the non-availability at our institute.

After conducting the survey among paramedical staff, the score found was above average. However they agreed that the device was user friendly and procedure was easy to learn and perform. As well as interpretation of results was quite easy and chances of injury in

Comparision with other conventional method were negligible.

In our study, we also found that out of 15 staff, some of them were more comfortable using slide, it maybe because they were trained and were using conventional methods (preferably slide method) for many years. So they were more accustomed to these methods and therefore it takes much less time for them to perform. This familiarity and accustomisation to slide method had resulted in resistance to accept this new technique. However in rural areas this method can be helpful as this new technique can be easily performed recalled and can be interpreted easily. This method also requires minimal training to the staff as compared by conventional method of blood grouping. In 2018, Tiwari AK, Setya D, Aggarwal G et al conducted a study on time taken by Erycard 2.0, after which he concluded that mean time taken by Erycard was 5.13 minutes and that by slide was 1.7 minutes.[1] In the present study, time taken by Erycard is more because as per the manufacturer's instruction, there is a time period of 3 minutes for which we have to wait after addition of buffer, for interpretation of result. However we also found out that as soon as the buffer reagent was added, within few seconds, the result was read. The mean time taken by Erycard was 2 min 8 sec, which was comparable to that of slide.

According to study conducted by Bienek DR, Chang CK, Charlton DG in 2009 on "Stability of user-friendly blood typing kits stored under typical military field conditions in which ABO-Rh combination blood typing experiment kit and Eldon home kit 2511 were used. In his study he showed that there were no discrepancies in the result in spite of exposure of kits to different temperature and manipulative storage conditions. These results are concordant to the results obtained in the present study. In our study it was observed that inspite of exposing the devices in different temperatures for 15 days, no deviation of results were observed in all the tested devices when compared with the initial results.[8]

Tiwari AK, Setya D, Aggarwal G et al in his study also concluded that there were no deviation of results on device stability even if the kits were exposed to unfavorable temperature and humidity conditions.[1]

Hence in our study we also found out that that the result can still be easily interpreted even after 2 months of usage, this can help in keeping the records of patients for longer time. Henceforth, stability serves as a key factor in rural settings as these kits may be exposed to unfavorable conditions while transporting, so these devices are user friendly which require minimum storage conditions and hence can be beneficial for larger scale grouping. Hence Erycard 2.0 can be used in place of conventional method especially in rural settings. Erycard 2.0 is much expensive than slide. This minor disadvantage with this is that if the delivery of therapeutic interventions may be enhanced and if these devices start getting manufactured in much larger scale maybe this can reduce the cost of cards. Another problem with this is, as these cards are made of plastic so these can become a huge burden on the waste management, there should be proper management of this waste. These cards are provided with reagent strips, so if the manufacturer somehow be able to recycle and reuse these cards just by changing the reagent strip then this may resolve the problem.

Conclusion

This study will help Erycard 2.0 to emerge as a new point of care device for ABO and Rh blood grouping. They can be considered for use in rural settings, health campsbedside blood campaign and resource constrained

setting and can be used in place ofconventional slide method due to its many advantages over slide method like better accuracy, stable results, stability of device in wide temperature variation, the risk of contamination of blood sample is much less than that of the slide method in off-site useand easier interpretation of the results. Moreover, the device is easy to use and doesn'trequire any extensive training to perform the ABO and Rh blood grouping and can be performed by any individual with minimal exposure to blood banking.

Limitations

The study is having a limitation of small sample size being a short-term studentship project to be completed within a small-time frame but the utility of the chromogenic media in resource poor settings needs to be explored in large scale and multi-centric trials for correct identification using less human hours and cost benefit.

Acknowledgment

We would like to acknowledge the ICMR for choosing the important topic under short term student ship program. We would like to extend our thanks to Mr. Banwari and Mr. Jitendra, Clerk in MCI cell, for all the technical and official helps extended in carrying out the study.

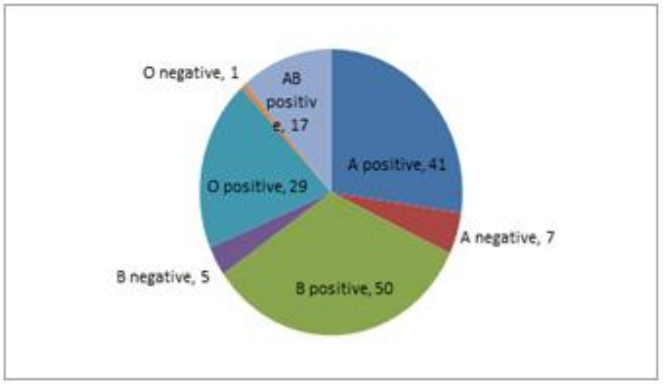
Financial support and scholarship: This study was self-funded with grant from STS-ICMR and carried out by the department of Pathology, Raipur Institute of Medical Sciences, off NH-6, Bhansoj Road, Gram Godhi, Raipur – 492101, Chhattishgarh, India.

References

1. Tiwari AK, Setya D, Aggarwal G, Arora D, Dara RC, Ratan A, et al. Evaluation of new indigenous "point-of-care" ABO and Rh grouping device. J Lab Physicians 2018; 10:80-4.
2. Ransohoff JR, Melan son SEF. What's New in Point-of-Care Testing? Point of Care 2019; 18:92–9.
3. Dhruva G, Agra vat A, Bhankhodia V. Comparison of conventional Tube agglutination method versus Erycard 2.0 for the ABO blood grouping system – A pilot study. Int J Res Med 2015; 4:59-61.
4. Mujahid A, Dickert FL. Blood group typing: From classical strategies to the application of synthetic antibodies generated by molecular imprinting. Sensors 2015, 16:51.
5. Yousuf R, Abdul Ghani SA, Abdul Khalid N, Leong CF. Study on ABO and RhD blood grouping: Comparison between conventional tile method and a new solid phase method (Intec Blood Grouping Test Kit). Malaysian J Pathol 2018; 40(1): 27–32
6. Erycard 2.0 user guide accessed on http://www.tulipgroup.com/Tulip_New/html/pack_inserts/ERYCARD_2.0.pdf
7. ABO grouping and Rh typing accessed onhttp://nbtc.naco.gov.in/assets/resources/training/5.pdf
8. Bienek DR, Chang CK, Charlton DG. Stability of user-friendly blood typing kits stored under typical military field conditions. Mil Med. 2009 Oct;174(10):1075-80.

Legend Graphs and Tables

Graph 1: Incidence pattern of different blood groups



Graph 2: Average time taken for blood grouping

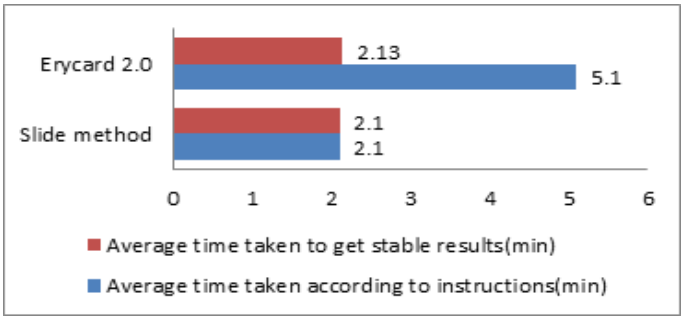


Table 1: Comparison of sensitivity of Erycard 2.0 over slide and tube agglutination technique

	Erycard 2.0	Slide method
Concordant results	147/150(98%)	147/150(98%)
Discordant results	3/150(2%)	3/150(2%)

Table 2: Types of discrepancies

Sample number	Correct blood group (by tube method)	Blood group by Erycard 2.0	Blood group by slide agglutination method	Type of discrepancy	Reason/ Remark
24	AB positive	A positive	A positive	ABO	Low antigenic expression
57	B positive	O positive	O positive	ABO	
120	A positive	O positive	O positive	ABO	

Table 3: Comparison of time taken by Erycard and slide method for blood grouping

Sample number	Time taken by slide method	Time taken by Erycard 2.0	
		According to instructions	To get stable result
1.	2 min 14 sec	5 min 9 sec	2 min 14 sec
2.	1 min 54 sec	4 min 57 sec	2 min 20 sec
3.	2 min 10 sec	5 min 14 sec	2 min 9 sec
4.	2 min 4 sec	5 min 18 sec	1 min 58 sec
5.	2 min 17 sec	5 min 7 sec	2 min 23 sec
6.	1 min 46 sec	4 min 53 sec	1 min 48 sec
7.	2 min 8 sec	4 min 55 sec	2 min 21 sec
8.	2 min 24 sec	5 min 17 sec	2 min 4 sec
9.	2 min 7 sec	5 min 13 sec	2 min 6 sec
10.	1 min 54 sec	4 min 54 sec	1 min 55 sec
AVERAGE	2 min 6 sec	5 min 6 sec	2 min 8 sec

Table 4: Score for ease of use of Erycard 2.0

Question	Total score(n=15)	Mean score
Is Erycard 2.0 easy to learn and recall?	49	3.26
Is Erycard 2.0 easy to perform?	46	3.06
Is Erycard 2.0 user friendly?	57	3.8
Is it easy to interpret results in Erycard 2.0?	47	3.13
Would you prefer Erycard 2.0 over slide method?	52	3.46