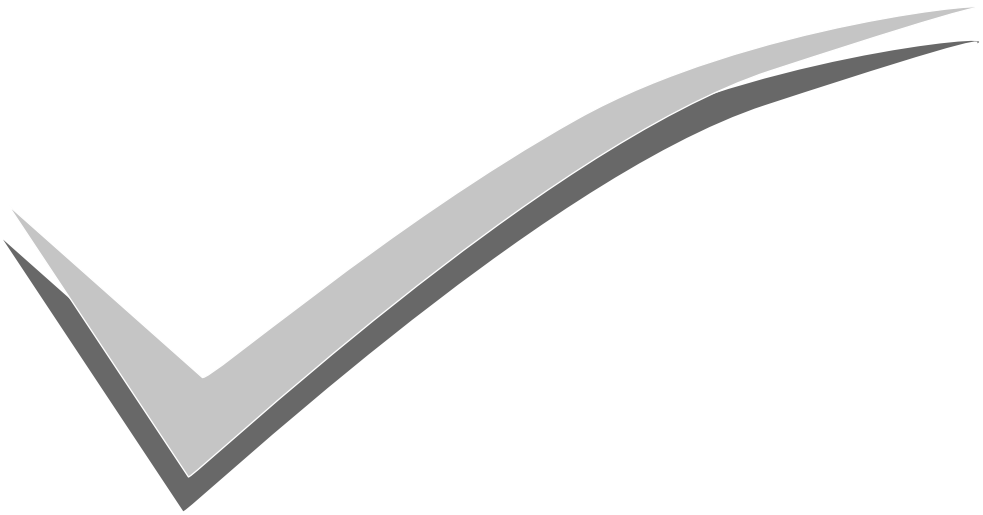
CE

ISO 13485:2016

Performance Evaluations



ERYCARD 2.0™

Blood Grouping Card for ABO/Rho(D) Forward Grouping with Autocontrol

Performance Evaluations

CE

ISO 13485:2016

INDEX		
S. No.	**Name of the Publication**	**Pg Nos**
1.	Journal of Laboratory Physicians. 2018 Jan-Mar; 10(1)	80-84
2	Int J Res Med. 2015; 4(1) e ISSN:2320-2742 p ISSN: 2320-2734	59-61

ERYCARD 2.0™

Blood Grouping Card for ABO/Rho(D) Forward Grouping with Autocontrol

Performance Evaluations

CE

ISO 13485:2016

OTHER EVALUATIONS

INDEX	
S. No.	**Name of the Evaluating Body**
3.	Bharat Vikas Parishad Pathology Laboratory, Pune, India
4.	Internal Evaluation, Tulip Diagnostics (P) Ltd, Goa

ERYCARD 2.0™

Blood Grouping Card for ABO/Rho(D) Forward Grouping with Autocontrol

Journal of Laboratory Physicians
Official Publicationn of the Indian Association of Laboratory Physicians (IALP)
Home
Current issue
Instructions
Submit article

J Lab Physicians. 2018 Jan-Mar; 10(1): 80–84.
doi: 10.4103/JLP.JLP_71_17
PMCID: PMC5784300
PMID: 29403211

Evaluation of new indigenous “point-of-care” ABO and Rh grouping device

Aseem Kumar Tiwari, Divya Setya, Geet Aggarwal, Dinesh Arora, Ravi C. Dara,[1] Ankita Ratan, Gunjan Bhardwaj, and Devi Prasad Acharya

Department of Transfusion Medicine, Medanta - The Medicity, Gurgaon, Haryana, India
[1]*Department of Transfusion Medicine, Manipal Hospitals, Jaipur, Rajasthan, India*
Address for correspondence: Dr. Aseem Kumar Tiwari, Department of Transfusion Medicine, Medanta - The Medicity, Sector-38, Gurgaon - 122 001, Haryana, India. E-mail: aseemtwr@yahoo.co.in

Received 2017 Apr 12; Accepted 2017 Jul 21.

Abstract

Go to:

BACKGROUND:

Go to:

Erycard 2.0 is a “point-of-care” device that is primarily being used for patient blood grouping before transfusion.

MATERIALS AND METHODS:

Go to:

Erycard 2.0 was compared with conventional slide technology for accuracy and time taken for ABO and Rh forward grouping result with column agglutination technology (CAT) being the gold standard. Erycard 2.0 as a device was also evaluated for its stability under different storage conditions and stability of result till 48 h. In addition, grouping of hemolyzed samples was also tested with Erycard 2.0. Ease of use of Erycard 2.0 was evaluated with a survey among paramedical staff.

RESULTS:

Go to:

Erycard 2.0 demonstrated 100% concordance with CAT as compared with slide technique (98.9%). Mean time taken per test by Erycard 2.0 and slide technique was 5.13 min and 1.7 min, respectively. After pretesting storage under different temperature and humidity conditions, Erycard 2.0 did not show any deviation from the result. The result did not change even after 48 h of testing and storage under room temperature. 100% concordance was recorded between pre- and post-hemolyzed blood grouping. Ease of use survey revealed that Erycard 2.0 was more acceptable to paramedical staff for its simplicity, objectivity, and performance than conventional slide technique.

CONCLUSION:

Go to:

Erycard 2.0 can be used as “point-of-care” device for blood donor screening for ABO and Rh blood group and can possibly replace conventional slide technique.

Keywords: ABO grouping, Column Agglutination Technology, donor screening, ease of use, Erycard, lateral flow, point of care, stability

Introduction

Go to:

The basic serological technique in any blood transfusion service is ABO and Rh grouping, the principle of which is based on specific agglutination reaction between antigen on red cells and antibodies in the serum. ABO blood grouping is done in two steps; first is the red cell typing or forward grouping and

the second step is the serum or reverse grouping. However, Rh grouping is done in a single step, that is, forward grouping.

There is a wide range of various analytical tests available for ABO and Rh blood group typing. Some are age old classical ones such as tube or slide tests, whereas some are relatively modern day methods such as solid-phase red-cell adhesion and column agglutination technology (CAT).

Grouping by slide method has a lot of limitations. It has been proved that slide grouping should always be supplemented with a more robust grouping technique comprising both cell and serum grouping.[1] Some of the limitations of slide method include drying up of reaction mixture, difficulty in interpreting weaker reactions, mixing up of reaction mixtures, misinterpretation due to inadequate mixing of RBC and antisera, no reproducibility, and many others.[2] Despite being less sensitive, it is still used as preliminary and usually point-of-care (POC) technique because of its simplicity and ease of use, especially in resource-constrained settings.[3] Recently, a new indigenous POC device Erycard 2.0 has been introduced for determining ABO and Rh blood groups which is based on the principle of lateral flow guided by capillary action. 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.

This study was undertaken with an aim to evaluate and compare the accuracy of Erycard 2.0 against conventional slide technique with CAT as the gold standard. In addition, ease of use, grouping of hemolyzed samples, stability of the device, and stability of the results given by Erycard were also tested.

Materials and Methods

Go to:

Settings and design

This was a prospective, analytical study performed at a tertiary health-care-based blood bank on blood donors from July to August 2016. The blood bank collects around 25,000 whole blood units annually.

Erycard™ 2.0 blood grouping test Erycard 2.0 blood grouping card for ABO and Rh(D) forward grouping with autocontrol is based on the principle of lateral flow. It is a POC device manufactured by Tulip Diagnostics Ltd., Goa, India. Using the fixed volume micropipette provided, 5 μl of test participant's whole blood sample was added to each of the 4 wells, ensuring that only the blood drop was in contact with the reagent. After 1 min, two drops of buffer were added to each well. After waiting for 3 min, the results were interpreted. The autocontrol should always show a colorless patch for valid interpretation.

Conventional slide grouping On a clean slide, one drop of Anti-A, Anti-B, and Anti-D were taken, and three drops of blood were added to the drop of antisera. Each solution was mixed carefully with a separate applicator stick. The slide was rocked back and forth slowly for around 1 min and then agglutination was recorded.

Automated Column Agglutination Technology CAT was considered as the gold standard method for blood grouping. Blood group for all donors was performed by automated CAT-based equipment (AutoVue Innova, Ortho Clinical Diagnostics, UK). This technology is objective, sensitive, straightforward, and relatively easy to operate.

Comparison of blood grouping between slide and Erycard 2.0 For comparison of accuracy of blood grouping between slide and Erycard 2.0

This comparison was performed on 550 consecutive blood donors. Using a single fingerprick, capillary blood sample was taken for grouping by slide and Erycard. Grouping by CAT in AutoVue was done from the venous sample obtained from the donor at the time of donation. All samples whose results were concordant on slide, Erycard and AutoVue grouping were considered correct. For samples, where there was discordance between Erycard 2.0 and slide; AutoVue result was considered final.

For comparison of time span for slide grouping and grouping by Erycard 2.0

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

Other evaluations of Erycard 2.0 Assessing the effect of temperature and humidity on the devices

To study the effect of storage, temperature, and humidity conditions, 24 devices each were kept in four different environmental setups for 30 days and then tested simultaneously. The four setups included high temperature with high humidity, high temperature with low humidity, low temperature with low humidity, and low temperature with high humidity. A control group of 24 devices was also kept at the optimum temperature (2°C–30°C), as described in the manufacturer's instructions. The humidity for control was maintained between 30% and 35%.

In all the settings, the container and thermohygrometer were checked every day for 30 days. The cards were taken out on the 31st day. Using 24 known donor blood samples (containing both Rh D positive as well as Rh D negative samples), blood grouping was performed on devices kept in setting 1,2,3,4 and control simultaneously. The results were recorded and compared.

Setting 1: High temperature with high humidity.

A dry incubator was set at 45°C. Open containers filled with water were placed on all shelves. A thermohygrometer was placed inside the incubator to record the temperature and humidity. The devices were placed in the incubator. The humidity was maintained between 70% and 75%.

Setting 2: Low temperature with high humidity.

Twenty-four devices were placed in a container with open surface in the cold room. A thermohygrometer was placed inside the container to record the temperature and humidity. The temperature ranged between 4°C and 6°C, and humidity was maintained between 80% and 85%.

Setting 3: High temperature with low humidity.

Twenty-four devices were placed in a container with the thermohygrometer, and the temperature was set at 45°C. The humidity was maintained between 10% and 15%.

Setting 4: Low temperature with low humidity.

An airtight container was taken and kept inside the incubator, when warm it was taken out and silica gel was placed inside it along with 24 devices and the thermohygrometer. The container was closed immediately and was wrapped with cellophane tightly. The container was transparent, and the thermohygrometer was placed in such a position that it could be read at any time. This setup was placed in the cold room at 4°C–6°C, and the humidity was maintained between 30% and 35%.

Assessing stability of results in Erycard 2.0

To test the stability of the results obtained by Erycard 2.0, blood grouping of unknown fifty donor samples was performed. The initial results were recorded, and this was considered as 0 h. The devices were left at room temperature and interpreted after every 6 h. The interpretations were recorded at the end of every 6 h, and this was done till 48 h after which the devices were discarded.

Assessing the effect of hemolysis on the accuracy of blood grouping by Erycard 2.0

To test the effect of hemolyzed samples on the accuracy of the device, blood grouping of known samples (5 each of A positive, A negative, B positive, B negative, AB positive, AB negative, O positive, and O negative) was performed by personnel 1. After recording the blood groups, samples were centrifuged, plasma was removed, and distilled water was added to the red cells and centrifuged again. After centrifugation, the supernatant was checked for hemolysis, and hemolyzed samples were mixed thoroughly before performing blood grouping. Blood grouping using Erycard 2.0 was performed on these hemolyzed samples and recorded by personnel 2.

Survey for “ease of use” of Erycard 2.0 A survey was conducted for 28 paramedical staff working in blood bank including nursing staff and laboratory technicians to assess the acceptance of Erycard 2.0 over slide method. The questionnaire had four questions and was a 4-point Likert scale. All participants

were explained the technique and were asked to perform the same on unknown samples. After performing the test, they were asked to fill up the questionnaire individually.

Statistical analysis

Differences in the discordant grouping results between conventional slide grouping, and blood grouping by Erycard were analyzed and sensitivity and specificity for the new method were calculated.

Ethics Committee approval

The 20 µl whole blood sample that was required for Erycard 2.0 blood group testing was obtained from the same finger prick as the sample for slide grouping; additional prick was not done. Since no donor discomfort was involved in acquiring the sample, hence the institution waivered off the consent and ethical approval.

Results

Go to:

Comparison of blood grouping between conventional slide method and Erycard 2.0 was performed on two parameters; accuracy of result on 550 blood donors and time span to result on additional 50 blood donors. Evaluation of Erycard 2.0 was performed on four parameters; effect of temperature and humidity on 96 devices, stability of results was studied on 50 devices, effect of hemolysis on accuracy of blood grouping on 40 devices, and a survey for ease of use was also conducted.

For comparison of blood grouping between slide and Erycard 2.0

Comparison of accuracy of blood grouping between slide and Erycard 2.0 A total of 550 healthy, volunteer blood donors were tested by both conventional slide grouping and by Erycard 2.0 and compared with CAT (gold standard).

Concordant results were obtained in 544/550 (98.9%) samples. Out of the six discrepancies that occurred, none were given by Erycard. The positive predictive value of Erycard was 100% and sensitivity was 100% [Table 1]. Out of the six discrepancies, one was an ABO discrepancy, whereas five were Rh discrepancies [Table 2].

Table 1

Comparison of accuracy of blood grouping between slide and Erycard 2.0

	Conventional slide grouping (%)	Erycard 2.0 grouping (%)
Correct result	544 (98.9)	550 (100)
Incorrect result	6 (1.09)	0
Total	550	550

Table 2

Types of discrepancies

Sample number	Group by slide	Group by Erycard	Group by CAT	Type of discrepancy
47	A positive	O positive	O positive	ABO
179	B negative	B positive	B positive	Rh
268	O positive	O negative	O negative	Rh
392	AB negative	AB positive	AB positive	Rh
438	A positive	A negative	A negative	Rh
475	B positive	B negative	B negative	Rh

CAT = Column agglutination technology

Comparison of time span for slide grouping and grouping by Erycard 2.0 Time taken to perform blood grouping on Erycard 2.0 and slide method was recorded using a stopwatch on fifty samples. The mean of the time taken was calculated [Table 3].

Table 3

Comparison of time span for slide grouping and grouping by Erycard 2.0

Technique	Mean time taken (min)	Range (min)
Slide	1.7	1.43-1.93
Erycard 2.0	5.13	4.67-5.77

Evaluation of Erycard 2.0

Assessing the effect of temperature and humidity on the devices The devices stored at four different environmental conditions for 30 days each showed that there is no effect of temperature and humidity variations on the accuracy of blood grouping by Erycard.

Assessing the stability of results obtained by Erycard 2.0 All fifty devices showed no deviation from the initially observed result at 6 h intervals till 48 h.

Assessing the effect of hemolysis on the accuracy of blood grouping by Erycard 2.0 All 40 tests showed the same blood group before and after hemolysis.

Survey for ease of use of the new device

Twenty-eight paramedical staff of blood bank participated in the study. On the basis of the responses obtained from the questionnaires, the mean score for each question was calculated [Table 4].

Table 4

Survey for ease of use of the new device

Question	Total score (*n*=28)	Mean score
Grouping by device is easy to learn, recall, and perform	96	3.42
Grouping by device is easy to interpret	100	3.57
Grouping by device is user-friendly	96	3.42
Preference of device over slide grouping with respect to tidiness	97	3.46
Preference of device over slide grouping with respect to drying of reaction mixture	97	3.46
Preference of device over slide grouping with respect to lesser chances of sharp injury	100	3.57

Discussion

Go to:

Even today, several blood banks in India use slide grouping as a preliminary method for blood grouping. At present, several POC devices are available for forward grouping which are being used for bedside grouping of patients, but these devices can be used in donor screening as well. POC testing for ABO and Rh blood group finds use in the primary labeling of blood bags at the time of donation which is necessary for maintaining the inventory and also as a check for the final labeling of blood bags. Furthermore, initial blood grouping is important when looking for same blood group donors to perform plateletpheresis and for buffy coat pooling.

The present study was conducted to evaluate Erycard 2.0 as a blood grouping test for blood donor screening. The results from this study demonstrate that ABO and Rh determination with a simple POC device is easy and accurate. Although slide grouping is still used at many centers, it has a lot of drawbacks and Erycard 2.0 can replace grouping by slide in places where grouping might help decrease the errors leading to mismatched blood transfusion.

In the present study, the device demonstrated 100% concordance with CAT, the gold standard. In 2015, El Kenz and Corazzatested a POC ABO agglutination test device and observed that there was 100% concordance between the POC testing device and their laboratory instruments.[4]

However, Dhruva *et al*.[3] conducted a study in 2015 on the accuracy of Erycard 2.0, after which they concluded 97.6% concordance between results obtained by Erycard and that by their gold standard (conventional tube technique).[5] They found 7/300 discrepancies in patient samples tested and the discrepancies were due to low hematocrit (<15%), autoclumping, anti-A1 antibody, and hemolyzed sample. However, since the present study was conducted on donor sample obtained from a fingerprick, the above-mentioned causes of discrepancy were not pertinent to the present study.

The device is designed as a POC test to be used with freshly obtained whole blood. The evaluation of Erycard 2.0 was done using whole blood from a fingerprick. This was an advantage over the study conducted by Thomas Herold *et al*., who performed their testing on previously collected stored samples.[6] Hemolysis and sample degradation could result from handling variations and prolonged storage and thus cause deviation in results.

In the present study, there was significant difference in the average time taken for blood grouping by Erycard 2.0 and by the slide. Although the time taken by Erycard was more, the method was less messy and more objective as compared to slide method of blood grouping.

In 2009, Bienek *et al*. conducted a study to test the stability of user-friendly blood typing kits stored under typical military field conditions[7] Eldon Home Kit 2511 (Eldon Biologicals A/S, Denmark) and ABO-Rh Combination Blood Typing Experiment Kit (Lab Aids, Inc., NY, USA) were used. No differences were found between results from kits stored under manipulated storage conditions and those stored at optimum storage conditions. These results were similar to the results obtained in the present study, which indicate that during transportation, even if the devices are exposed to unfavorable

temperature and humidity conditions, the accuracy of blood grouping obtained by Erycard 2.0 is not affected.

In the present study, no deviations were observed in all the tested devices from the initial result, till 48 h after testing. As per the manufacturer's instructions, for stable results, the devices must be stored in a sealed cover without contamination in a cool, dry place, and avoid exposure to direct sunlight and heat. The tested devices in the present study were left open at room temperature which is maintained between 20°C and 24°C normally. This observation is important when results need to be stored to solve blood grouping discrepancies while labeling of blood bags and also when donors come to blood banks with doubts about their blood group.

Hemolyzed samples may produce erroneous results of many laboratory tests including blood group testing. Supernatant hemoglobin can produce discrepancies between forward and reverse group. Hence, determining the exact blood group of hemolyzed samples is difficult. In the present study, it was observed that Erycard 2.0 determines all blood groups (A positive, A negative, B positive, B negative, AB positive, AB negative, O positive, and O negative) of hemolyzed samples correctly. This observation is extremely important since with even the most sensitive techniques, sometimes transfusion services are unable to comment on the blood group of hemolyzed samples.

The results obtained from the survey conducted for the paramedical staff suggested that staff agreed that the new device is easy to learn, recall, perform, interpret, and is a user-friendly. The staff preferred Erycard 2.0 over slide grouping due to its tidiness, no drying of the reaction mixture, and less chances of sharp injury.

Conclusion

Go to:

Erycard 2.0 is easy to use and interpret and even with minimal training blood bank staff can perform blood grouping easily. The device can become a useful tool for determining blood group of hemolyzed samples. Overall accuracy of the device is better than slide technique and hence can be used as a method of preliminary blood group testing.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

Acknowledgment

Go to:

The authors would like to acknowledge Tulip Diagnostics Private Limited, Goa, India, for providing devices free of cost.

References

Go to:

1. Prasad JP. Guidance Manual on “ABO and Rh Blood Grouping”. Document ID No. NIB/BRL/GM/02. National Institute of Biologicals (Ministry of Health & Family Welfare) Government of India. 2013:10–2.

2. Mujahid A, Dickert FL. Blood group typing: From classical strategies to the application of synthetic antibodies generated by molecular imprinting. Sensors. 2015;16:51. [PMC free article] [PubMed]

3. Malomgré W, Neumeister B. Recent and future trends in blood group typing. Anal Bioanal Chem. 2009;393:1443–51. [PubMed]

4. El Kenz H, Corazza F. Automated point-of-care testing for ABO agglutination test: Proof of concept and validation. Vox Sang. 2015;109:79–85. [PubMed]

5. Dhruva G, Agravat 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.

6. Herold TJ, Whittaker DS, Glynn T. Determining the accuracy of a rapid point-of-care test for determining Rh(D) phenotype. Acad Emerg Med. 2005;12:474–6. [PubMed]

7. Bienek DR, Chang CK, Charlton DG. Stability of user-friendly blood typing kits stored under typical military field conditions. Mil Med. 2009;174:1075–80. [PubMed]

ORIGINAL ARTICLE

Comparison of conventional TUBE agglutination method versus ERYCARD™2.0 for the ABO blood grouping system-A Pilot Study

Gauravi Dhruva[1*], Amit Agravat[2], Vaishali Bhankhodia[3]

[1]Professor & Head, [2]Associate Professor, [3]1st Year Resident, Department of Pathology, P. D. U. Govt. Medical College – Rajkot (Gujarat)

ABSTRACT

BACKGROUND: Aim of our study was to compare the ease of use and accuracy of conventional tube agglutination method versus ERYCARD™2.0. **MATERIALS AND METHODS:** 300 anticoagulated blood samples from patients were collected & submitted to Clinical OPD Laboratory at P.D.U. Medical College and Hospital-Rajkot from December 2013 and January 2014. Sample selection was purposely biased toward those from anemic or those with autoagglutination. All blood samples were tested by use of tube agglutination & ERYCARD™2.0. **RESULTS:** Total number of samples received in our department was 300 out of 7 samples in which blood grouping discrepancies arose with ERYCARD™2.0. **CONCLUSION:** Compared with the historical gold-standard TUBE Agglutination method and excellent agreement was achieved with erycard™2.0, by this method provides simple and accurate typing for the ABO blood group system with few discrepancies. Retyping after typing with TUBE laboratory methods is recommended to confirm.

Keywords: Blood grouping, Erycard™2.0, Tube Agglutination Method.

INTRODUCTION

ABO RH grouping system is the most important test in medical laboratory and blood banking system, performed both on transfusion recipients and blood donors. The critical nature of ABO grouping stems from two characteristics of the system. First, unlike other blood group systems, antibodies of the ABO system are present in the serum of almost every person who does not have the corresponding antigen. ([5]) Second, 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. While the cross-match affords an additional measure of protection, this may not be done in every case.

Accurate determination of a person's ABO group requires two different test procedures: red cell grouping also called as forward grouping and serum grouping also called as reverse grouping. The individual is first assigned to one of the four ABO blood groups - A, B, AB and O based on the reaction of red cells with blood grouping sera Anti-A and Anti-B. Anti AB serum prepared from specially selected group O individuals is not a simple mixture of Anti-A and Anti B but is the component of group O serum that has the special property of reacting with weak antigens on the red cells, especially weak A antigens. Blood group is detected by various methods. ERYCARD™2.0 is blood grouping card easy to use in bed site setting or outdoor camp and easy to interpret by laboratory staff. TUBE agglutination is gold standard method for ABO blood grouping as this method gives incubation time. Therefore, the aim of thevpresent study to compare ease of use and accuracy of ERYCARD™ 2.0 with vgoldstandard TUBE agglutination method.

Patients: It was a cross-sectional study done on routine samples over a period of 2 months December 2013 and January 2014. Total numbers of 300 patients were randomly investigated in OPD laboratory.

Blood samples: Under all aseptic precautions, samples were collected from the antecubital vein using a 2-ml disposable syringe with 24G needle. The

***Corresponding Author**
Dr. Gauravi A. Dhruva
32, Suraj Apartment-1,
Shroff Road, Rajkot – 360001
Email: gauravidhruva@yahoo.co.in

study included small (1- to 2-mL) EDTA anticoagulated blood samples from patients.

MATERIALS AND METHODS

CARD method: In this study ERYCARD™2.0 blood grouping card for ABO/Rho (D) forward grouping with autocontrol is used , which is based on principle of lateral flow guided by capillary action. Procedure: Bring the pouch and reagent buffer bottle to room temperature. Tear open the pouch just prior to the testing and remove the ERYCARD™2.0 test device. Label the test card with the patient's ID and date. Add 5µl each of the patient's whole blood sample to each of the sample wells indicated as 'S', ensuring that only the blood drop is in contact with pre-dried reagent on the sample pad and adsorbed by it. In case the micropipette tip touches the sample pad, discard the tip and use fresh tip for dispensing into next sample well. After waiting for one minute allowing the sample to react with the reagent on sample pad adds two drops of the reagent buffer to each of the reagent wells indicated as 'R'. After addition of reagent buffer wait for 3minutes to interpret the test results.[2] The auto control should show a colorless patch before the results can be interpreted correctly. If the autocontrol pad has a color then the test result should not be interpreted.

TUBE method: to prepare a RBC suspension for the TUBE method, 1 ml of anticoagulated blood was centrifuged for 2 minutes in a centrifuge (1,000 × *g* at Room temperature [approx 20°C]). Plasma was collected into a separate tube, and the RBC Pellet was resuspended in 5 ml of normal saline. The suspension was then recentrifuged and resuspended 3 times and finally reconstituted to a 2% to 5% RBC suspension. In 3 tubes, 25µL of this suspension was then mixed with 50 µL of antiserum. These mixtures were incubated at room temperature for 15 minutes before centrifugation for 15 seconds at 1,000 × *g*.[1] Tubes were then gently agitated, and the degree of agglutination was scored. Interpretation of the test [4]

4+ cell button remains in one clump.

3+ cell buttons dislodges into several clumps.

2+ cell buttons dislodges into many small clumps of equal size.

1+ cell button dislodges into finely granular, but definite, small clumps.

D cell button dislodges into fine granules, but not definite small clumps. Results should be recorded as doubtful.

0 Negative reaction-cell buttons dislodges into no visible clumps.

RESULT

Blood samples from 300 patients were included in the study. All the patients were tested by both blood grouping methods. The strengths of all test reactions (anti-A, anti-B, and anti-D) were recorded as well as the

Interpreted test result for both methods. Accuracy of test methods was then calculated by comparison with the TUBE method as the standard criterion. Overall agreement between blood-typing methods was good to excellent, with identical results obtained in 293 of 300 (97.6%) patients tested with card. Details of the 7 discrepancies identified among these patients were summarized

DISCORDANT RESULTS

Number of samples	Correct blood group	Blood group by erycard™ 2.0	Blood group by tube agglutination method	Remark / reason
2	AB	O	AB	Hct < 15% [Anemia]
2	B	AB	B	Auto clumps
	O	AB	O	
2	A_2	O	A_2	Anti-A1 serum
	A_2B	B	A_2B	
1	O	-	-	Sample Hemolysed

Among the 300 samples examined in this study, there were 7 samples in which blood typing problems or discrepancies arose. Two of these samples were from Patients that had a recorded diagnosis of an anemia on the basis of an Hct < 15%. While the TUBE assay identified these samples to be blood type AB, card were falsely identifying these patients as blood group O. In addition, 2 of 7 samples that had auto clumps present. In this patients card showing weakly AB Positive group falsely, actually 1 of this 2 patients had B positive group and 1 patient had O positive group. Weak auto agglutination which was eliminated by washing of RBCs. 2 of 7 samples had A_2 or A_2B group that were not detected by card method, were

detected by tube method by comparing clump size of A with O agglutination and confirmed by anti A_1 serum.1 of 7 samples were hemolysed.

DISCUSSION

The ABO blood group system is the most clinically important blood group system in humans because ABO mismatched transfusions can cause life-threatening hemolytic reactions without prior sensitization via pregnancy or transfusion. Therefore, it is crucial that

Clinicians identify a human's blood group. In the present study, 2 ABO typing methods were compared for ease of use and accuracy. The TUBE tests are generally restricted to the laboratory and performed by specifically trained personnel, whereas the CARD is simple point-of care kits commonly used by laboratory staff in practice. Over the past 15 to 20 years, the reagents in blood-typing kits or tests have occasionally changed, and it is important to remember this when interpreting and comparing our results with those from previous Studies. In ERYCARD™2.0, the appropriate reagent are pre-dried at appropriate sample pad beneath the sample well namely Anti-A (IgM) antibodies in sample well A, Anti-B (IgM) antibodies in sample well B, Anti-D (IgM) antibodies in sample well D. The autocontrol is a negative control that does not contain any antibodies in sample well (Ctrl) and serve the validate the test results. Reagent Buffer contains sodium azide (<.1%) as a preservative. In TUBE method, Anti A, Anti B and Anti D reagent were ready to use reagent prepared from supernatants of mouse hybridoma cell cultures. These reagents contains sodium azide (<0.1%), sodium arsenite (0.02%) and bovine albumin. However, our survey also detected a few discrepancies, with the TUBE method 99% agreement and the ERYCARD™2.0 achieving

97.6% agreement. Therefore, CARD method should be suitable for point-of-care testing in in-clinic settings when typing results are immediately needed. Results of blood typing can be affected by anemia, auto agglutination and hemolysed sample, Thereby contribute to test inaccuracies as detected in the present study. In our study, blood samples from, anemic patients had positive results for the O antigen by ERYCARD™2.0.Preparation of appropriate concentration RBC suspensions alleviates the effect of Hct TUBE assays, and for the point-of-care assays, adding more blood to test reactions when dealing with anemic patients may overcome such problems. Subjective test interpretation is a potential problem with any of the methods used in our

Study but is of particular concern when agglutination is scored in an RBC suspension because test interpretation is dependent on the time of reading and degree of agitation applied by the operator. When the TUBE methods were used, the distinction between positive and negative results was clearer than those for the CARD method. This was because there were smaller numbers of 1+ and 2+ results with the TUBE methods, and such results may be confused, altering test interpretation.

CONCLUSION

Though ERYCARD™2.0 helped a lot in bedside blood grouping, on comparing the manual blood grouping methods, few discrepancies in blood grouping was noted. Card was easy to use and interpreted as compare to TUBE method but incubation could not be possible in this card method, as incubation possible TUBE agglutination method. Overall accuracy of blood group typing by ERYCARD™2.0 is as comparable as TUBE agglutination method, so it can be used as an optional method.

REFERENCE

1. Comparison of five blood-typing methods for the AB blood group system. Seth M, Jackson KV, Giger U
2. Humans Blood Groups by Geoff Daniels, Blackwell Science Ltd, 1995.
3. Mollison, P.L. Blood Transfusion in Clinical medicine:-10th Edition. Oxford: Blackwell Scientific Publication, 1997
4. Recommended Methods for blood Grouping Reagents Evaluation; Docket No. 845-0181
5. Importance of ABO Grouping.

Performance Evaluations

CE

ISO 13485:2016

OTHER EVALUATIONS

ERYCARD 2.0™

Blood Grouping Card for ABO/Rho(D) Forward Grouping with Autocontrol

Janakalyan Raktapedhi's
Bharat Vikas Parishad Pathology Laboratory

To,

Tulip Diagnostics Pvt.Ltd.

Please find attached the evaluation report on performance of ERYCARD™ 2.0. We have tested total number of 200 samples, out of which 200 were from healthy donors results of ERYCARD ™ 2.0 were compared with classical Tube Technique. There was 100% correlation between ERYCARD TM 2.0 and classical tube technique.

ERYCARD ™ 2.0 is easy to perform and interpretation of results is also simple. There was no person to person variation in interpretation of results. Because of its simple procedure and easy interpretation ERYCARD ™ 2.0 can be used for outdoor testing like outdoor blood donation camps, Blood grouping camps and patient bed side testing as pre transfusing testing.

Complete evaluation data is attached to this letter.

Dr.Amol Rajhans (M.D.Pathalogist)

Dr. Amol Rajhans
M.D. (Path), D.C.P.
Reg. No. 88539
Consulting Pathologist

Dr. Tushar Patil
M.B.B.S., D.C.P., D.N.B.(Path)
Reg. No. 075796
Consulting Pathologist

Dr. Atul Kulkarni
M.B.B.S., D.C.P. (Path)
Reg. No.2000/02/770
Consulting Pathologist

Janakalyan Raktapedhi, Pune, Regi. No. E 1226 (Pune)
1003, Shukrawar Peth, Near Swargate, Pune. **Ph - 2444 4502, 2444 9527.** E-mail : bvp.pathlab@gmail.com

ERYCARD 2.0 EVALUATION (External)

Name of the Institution:

Evaluation Date:

Kit Information:

Erycard 2.0: Rapid ICT for ABO and Rh screening

Lot No: Mfd date Exp date

Reference Kit:

	Lot No	Mfd date	Exp date
Anti-A	124214	Jun 2012	May 2014
Anti-B	124212	May 2012	Apr. 2014
Anti-D	124214	Jun 2012	May 2014
Novaclone ANTI D-NDMG06501			Dec. 2013

S.No.	Date	Patient ID	BLOOD GROUP		Comments
			Ref test (Method:	Erycard 2.0	
1		13175	B+ve	B+ve.	
2		13177	A+ve	A+ve.	
3		13180	O+ve	O+ve.	
4		13200	O+ve	O+ve.	
5		13203	O+ve	O+ve.	
6		13204	O+ve	O+ve.	
7		13205	A+ve	A+ve.	
8		13206	AB+ve	AB+ve.	
9		13207	O+ve	O+ve.	
10		13208	O+ve	O+ve.	
11		13209	B+ve	B+ve.	
12		13210	A+ve	A+ve.	
13		13211	A+ve	A+ve.	
14		13212	B+ve	B+ve.	
15		13213	O+ve	O+ve.	
16		13214	B+ve	B+ve.	
17		13215	O+ve	O+ve.	
18		13216	A+ve	A+ve.	
19		13217	O D4	O D4.	
20		13218	AB D4	AB D4.	
21		13219	O+ve	O+ve.	
22		13220	O+ve	O+ve.	
23		13221	B D4	B D4.	
24		13222	O+ve	O+ve.	
25		~~13223~~	~~A+ve~~		

Sr.no	Date	Patient ID	Blood Group		Comments
26			Ref test(Method :	Erycard 2.0	
27		13254	B +ve	B +ve	
28		13255	A +ve	A +ve	
29		13256	A +ve	A +ve	
30		13257	AB +ve	AB +ve	
31		13258	B +ve	B +ve	
32		13259	A +ve	A +ve	
33		13260	O +ve	O +ve	
34		13261	B +ve	B +ve	
35		13262	A +ve	A +ve	
36		13263	A +ve	A +ve	
37		13264	A +ve	A +ve	
38		13265	A +ve	A +ve	
39		13266	O +ve	O +ve	
40		13267	O +ve	O +ve	
41		13268	A +ve	A +ve	
42		13269	A +ve	A +ve	
43		13270	A +ve	A +ve	
44		13271	A +ve	A +ve	
45		13272	A DU	A DU	
46		13273	B +ve	B +ve	
47		13274	A +ve	A +ve	
48		13275	O +ve	O +ve	
49		13276	O DU	O DU	
50		13277	O +ve	O +ve	
51		13278	A +ve	A +ve	
52		13279	A +ve	A +ve	
53		13280	B +ve	B +ve	
54		13281	A +ve	A +ve	
55		13282	B +ve	B +ve	
56		13283	O +ve	O +ve	
57		13284	B +ve	B +ve	
58		13285	A +ve	A +ve	
59		13286	B +ve	B +ve	
60		13287	B +ve	B +ve	
61		13288	A +ve	A +ve	
62		13289	A +ve	A +ve	
63		13290	A +ve	A +ve	
64		13291	B +ve	B +ve	

65		13292	A +ve	A +ve	
66		13293	O +ve	O +ve	
67		13294	O DU	O DU	
68		13295	B +ve	B +ve	
69		13296	A +ve	A +ve	
70		13297	O +ve	O +ve	
71		13298	A +ve	A +ve	
72		13299	O +ve	O +ve	
73		13300	B +ve	B +ve	
74		13301	O +ve	O +ve	
75		13302	B +ve	B +ve	
76		13303	O +ve	O +ve	
77		13304	B +ve	B +ve	
78		13305	O +ve	O +ve	
79		13306	B +ve	B +ve	
80		13307	O +ve	O +ve	
81		13308	A +ve	A +ve	
82		13309	O +ve	O +ve	
83		13310	O +ve	O +ve	
84		13311	B +ve	B +ve	
85		13312	B +ve	B +ve	
86		13313	O +ve	O +ve	
87		13314	B +ve	B +ve	
88		13315	A +ve	A +ve	
89		13353	O DU	O DU	
90		13354	B +ve	B +ve	
91		13355	B +ve	B +ve	
92		13356	B +ve	B +ve	
93		13357	O +ve	O +ve	
94		13358	A +ve	A +ve	
95		13359	B +ve	B +ve	
96		13360	A +ve	A +ve	
97		13361	AB +ve	AB +ve	
98		13362	A +ve	A +ve	
99		13363	B +ve	B +ve	
100		13364	O +ve	O +ve	
101		13365	A +ve	A +ve	
102		13366	O +ve	O +ve	
103		13367	O +ve	O +ve	
104		13368	B +ve	B +ve	

105		13369	O +ve	O +ve	
106		13370	O +ve	O +ve	
107		13371	O +ve	O +ve	
108		13372	O +ve	O +ve	
109		13373	A +ve	A +ve	
110		13374	O DU	O DU	
111		13375	B +ve	B +ve	
112		13376	O +ve	O +ve	
113		13377	A +ve	A +ve	
114		13378	A +ve	A +ve	
115		13379	B +ve	B +ve	
116		13380	B +ve	B +ve	
117		13381	A +ve	A +ve	
118		13382	O +ve	O +ve	
119		13383	A +ve	A +ve	
120		13384	B +ve	B +ve	
121		13385	B +ve	B +ve	
122		13386	O +ve	O +ve	
123		13387	AB +ve	AB +ve	
124		13388	AB +ve	AB +ve	
125		13389	B +ve	B +ve	
126		13390	O DU	O DU	
127		13391	A +ve	A +ve	
128		13392	A +ve	A +ve	
129		13393	O +ve	O +ve	
130		13394	O +ve	O +ve	
131		13395	B +ve	B +ve	
132		13396	O +ve	O +ve	
133		13397	O +ve	O +ve	
134		13501	O +ve	O +ve	
135		13502	B +ve	B +ve	
136		13503	AB +ve	AB +ve	
137		13504	B +ve	B +ve	
138		13505	O +ve	O +ve	
139		13506	B +ve	B +ve	
140		13507	A +ve	A +ve	
141		13508	AB +ve	AB +ve	
142		13509	O +ve	O +ve	
143		13510	AB +ve	AB +ve	
144		13511	B +ve	B +ve	

145		13512	O+ve	O +ve	
146		13513	O+ve	O +ve	
147		13514	B+ve	B +ve	
148		13515	B+ve	B +ve	
149		13516	O+ve	O +ve	
150		13517	A+ve	A +ve	
151		13518	O+ve	O +ve	
152		13519	O+ve	O +ve	
153		13520	O+ve	O +ve	
154		13521	B+ve	B +ve	
155		13522	B+ve	B +ve	
156		13523	O+ve	O +ve	
157		1006	O+ve	O +ve	
158		1007	O +ve	O +ve	
159		1008	O +ve	O +ve	
160		1009	A DU	A DU	
161		1010	O DU	O DU	
162		1011	B+ve	B +ve	
163		1012	A+ve	A +ve	
164		1013	A+ve	A +ve	
165		1014	A+ve	A +ve	
166		1015	O +ve	O +ve	
167		1016	B+ve	B +ve	
168		1017	O+ve	O +ve	
169		1018	A+ve	A +ve	
170		1019	A+ve	A +ve	
171		1020	B+ve	B +ve	
172		1021	O DU	O DU	
173		1022	O+ve	O +ve	
174		1023	A+ve	A +ve	
175		1024	B+ve	B +ve	
176		1025	O+ve	O +ve	
177		13534	A+ve	A +ve	
178		13535	O DU	O DU	
179		13536	B+ve	B +ve	
180		13537	B DU	B DU	
181		13538	A+ve	A +ve	
182		13539	A+ve	A +ve	
183		13540	B+ve	B +ve	
184		13541	O+ve	O +ve	

185		13542	A +ve	A +ve	
186		13543	O +ve	O +ve	
187		13544	O +ve	O +ve	
188		13545	O +ve	O +ve	
189		13546	A +ve	A +ve	
190		~~1351~~			
191		1036	B+ve	B +ve	
192		1037	A DU	A DU	
193		1038	O +ve	O +ve	
194					
195					
196					
197					
198					
199					
200					
201					
202					
203					
204					
205					
206					
207					
208					
209					
210					
211					
212					
213					
214					
215					
216					
217					
218					
219					
220					
221					
222					
223					
224					

206		13524	AB +ve	AB +ve	
207		13525	O +ve	O +ve	
208		13526	B +ve	B +ve	
209		13527	B +ve	B +ve	
210		13528	AB +ve	AB +ve	
211		13529	B +ve	B +ve	
212		13530	B +ve	B +ve	
213		13531	B +ve	B +ve	
214		13532	A DU	A DU	
215		13533	AB +ve	AB +ve	

Performed By:

Date:

Doctor's Signature: Dr. Rajlaxmi A.K.

Seal of the Institution:

JKRP's Bharat Vikas Parishad
Pathology Laboratory
1003, Shukrawar Peth,
Saras Baug-Swargate Road,
Near Natraj Hotel, Pune-411002.

Instructions for use:

1) Do not use clotted samples
2) Mix samples properly before use
3) After adding sample to the sample port please wait for 2 mins before adding buffer (as weak samples take time to react)

TULIP DIAGNOSTICS (P) LTD.

ERYCARD™ 2.0 EVALUATION DATA

Internal Evaluation

In an internal evaluation for ERYCARD™ 2.0, we tested 500 samples randomly collected from pathology laboratories and Blood Banks. The results were confirmed by Classical Tube Technique and Matrix™ Gel System. ERYCARD™ 2.0 has shown 100% correlation with Classical Tube Technique and Matrix™ Gel System.

Group wise data of internal evaluation done at Tulip factory, Goa.

Blood Group	No. of Samples	ERYCARD™ 2.0	Classical Tube Technique	Matrix™ Gel System
A Positive	154	154	154	154
B Positive	115	115	115	115
AB Positive	38	38	38	38
O Positive	159	159	159	159
A Negative	12	12	12	12
B Negative	7	7	7	7
AB Negative	1	1	1	1
O Negative	14	14	14	14

External Evaluation

ERYCARD™ 2.0 was also evaluated by Janakalyan Raktapedi's, Bharat Vikas Parishad Pathology Laboratory, Pune. Evaluation was done against Classical Tube technique considering it as gold standard. The correlation between Classical Tube Technique and ERYCARD™ 2.0 was 100%.

External evaluation data – Janakalyan Raktapedi's, Bharat Vikas Parishad Pathology Laboratory, Pune - July 2011

Blood Group	No. of Samples	ERYCARD™ 2.0	Classical Tube Technique
A Positive	53	53	53
B Positive	54	54	54
AB Positive	11	11	11
O Positive	65	65	65
A Negative	4	4	4
B Negative	2	2	2
AB Negative	1	1	1
O Negative	10	10	10

The above data is based on the evaluation report given to us for ERYCARD™ 2.0 by the customer. Data on file Tulip Diagnostics Pvt. Ltd.

For, Tulip Diagnostics Pvt. Ltd

Authorized Signatory

TÜV ISO 9001

Corporate Office: Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex Post Office, Goa - 403 202, INDIA.
Tel.: (0832) 2458546 - 51, Fax: (0832) 2458544, E-mail: sales@tulipgroup.com, Website: http:// www.tulipgroup.com

Works: Plot Nos. 92/96, Phase II C, Verna Industrial Estate, Goa - 403 722, INDIA.
Tel : (0832) 6624555, Fax: (0832) 2783511. E-mail: tulipvrn@sancharnet.in

For further information contact :

TULIP DIAGNOSTICS (P) LTD

Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex Post Office, Goa - 403202, INDIA.

Tel.: +91 832 2458546-50 **Fax :** +91 832 2458544 **E-mail :** sales@tulipgroup.com **Website :** www.tulipgroup.com