VOLUME - XXII
ISSUE - CXXVIII
MAR/APR 2025

The Crux

BIMONTHLY FORUM FOR THE LABORATORIANS

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Editorial

In the evolving world of blood group serology, the Gel Test, developed by Lapierre, emerges as a ground breaking advancement. This innovative method addresses key challenges in standardization and offers enhanced sensitivity, specificity, and efficiency, making it accessible to laboratories of varying sizes and expertise. With the use of gel premixed with reagents, precise volumes, and a no-wash antiglobulin test that eliminates the need for resuspension of red cell buttons, the Gel Test minimizes the variation often encountered with traditional techniques.

The versatility of the Gel Test spans a wide range of crucial blood bank procedures, including antibody screening, antigen typing, and cross matching. What sets it apart is its simplicity these tests are easy to perform and provide clear, stable end points, which can be reviewed at a later time. The applications extend beyond routine tasks, potentially reaching into other areas of diagnostic testing, demonstrating its immense value in modern healthcare.

This issue of *The Crux* also delves into the Matrix Gel System from Tulip Diagnostics. This cutting-edge system is designed to handle all the aforementioned applications with precision and speed, underscoring the Gel Test's transformative role in blood testing. In addition, we present a series of fascinating case studies that illustrate the accuracy and rapidity of blood group detection using this technology.

We also provide insights into the CE Marking and Certification Process. This section explains how the CE mark indicates that a product complies with the stringent quality, performance, and safety standards set by the European Union a crucial consideration for laboratories and healthcare providers.

As always, we aim to enlighten and inform. We trust that as you explore the pages of this magazine, you will discover valuable knowledge that enriches your work and perspective.

Lastly, we haven't forgotten your well-being. Be sure to check out our special section, *Bouquet*, featuring content designed to stimulate both your mind and body. With a blend of fun exercises and thought-provoking insights, we hope to brighten your day.

Happy reading!

Gel technology

Gel technology is used for blood grouping, antibody screening and identification, compatibility testing, and other immunohematology applications. It provides standardized, efficient and reliable results compared to conventional methods.

GEL testing has a number of important advantages over routine TUBE testing, particularly when testing large numbers of samples. These include standardization, stability, and smaller sample volume, ease of performance, analysis, and rapidity.

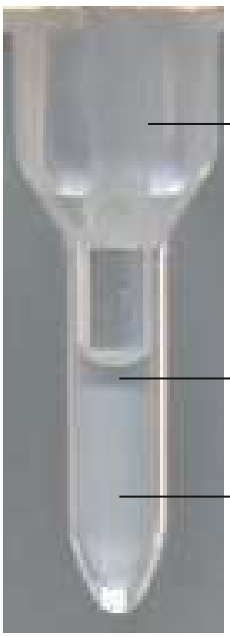
Gel technology in immunohematology is a method of testing blood that uses a gel column to analyze red blood cells (RBCs). It Gel Technology was born in 1985 in Lyon, France and was invented by Dr. Yves Lapierre.

The purpose of this technology was to minimize the problems associated with Classical Tube Technique like;

- Reduce False Negatives.
- Stabilize End Results.
- Easier, Convenient and Standardized procedure for Reproducible Results.

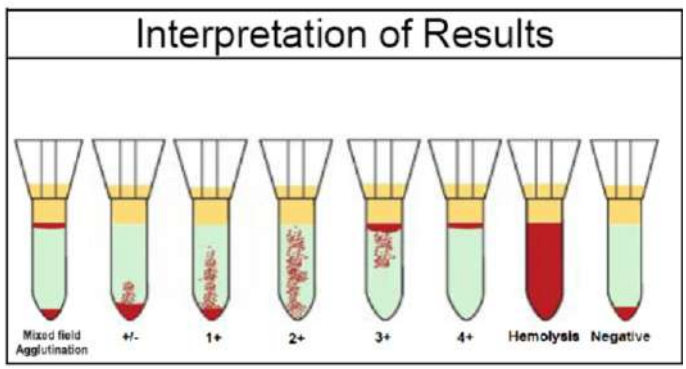
In Gel Technology the reaction is carried out in a specially designed card known as Gel card. This card consists of either six or eight microtubes. These microtubes are prefilled with gel and corresponding reagent (Anti-A, Anti-B, Anti-D, AHG) depending on the card parameter. The gel particles used are of different sizes and together acts as a sieve through which only a single normal RBC can pass through and represents a negative reaction. Agglutinated RBCs will be trapped in the gel column; their position in the gel column will depend upon the size of the agglutinate. The gel and the particle sizes will decide the sensitivity of the system. This technology is used to detect antibodies, determine blood types, and characterize autoantibodies.

Gel Card Microtube



A Gel Card

The red cell suspension is prepared using Low Ionic Strength Solution, Red cell suspension and serum/plasma is added to the reaction chamber of the microtubes. The gel card is then incubated and antigen antibody reaction takes place in reaction chamber. After incubation the gel card is centrifuged in card centrifuge as per standard centrifugal force with stated duration of time to the red cells in the microtube. Under these controlled conditions when a card is centrifuged only a single normal RBC can pass through the gel and settles at the bottom of the microtube forming a button. Reacted or agglutinated cells will be trapped on the gel column or in the gel column, their position in the gel will depend upon the size of the agglutinate and thus facilitates the grading of reaction.



In blood banks, commonly done tests are Blood Grouping and Crossmatching. Crossmatch or Compatibility test is one of the very important and skillful test of blood bank. This test is done prior to blood transfusion to check whether the donor's blood is compatible (matching) with the patient's blood. Routinely in blood banks this test is done by *Classical Tube Technique*. This technique is of *"Gold Standard"* in blood banking techniques. To get the desired outcome of this test should be done properly. This test is done by IAT method (Indirect Antiglobulin Test) which is Time consuming, Labour intensive and Skillful technique. A routine crossmatch done by Classical tube technique takes more than 1 hour and because of multiple steps involved in the test procedure the chances of errors are also increased. Classical tube technique also requires washing of red cells before and after incubation. Improper washing can neutralize the AHG reagent in the final steps of procedure and leads to false negative or down grading of results. Minimum of 30-45 minutes incubation at 37°C is required for complete reaction, reduction

in incubation time may lead to incomplete reaction and weak reaction can be missed. Because of labor intensive procedure of Classical tube technique many times the procedure of crossmatch is bypassed, either washing step is eliminated or incubation time is reduced or addition of Coombs control cells is skipped, which leads to improper test results, thus leading to immediate or delayed transfusion reaction to the patient.

Following are the problems often faced by the customer while doing crossmatch with Classical tube technique:

- **Washing Step**- Improper washing may lead to false negative or downgrading of results.
- **Cell Suspension or Ag-Ab ratio**- Correct concentration of cell suspension (3 or 5%) is required to maintain Ag-Ab ratio.
- **Normal Saline**- Normal saline increases the electron cloud around the red cells and slows down the Ag-Ab reaction. pH of Normal saline is also very important.
- **Incubation Time**- A minimum 30-45 minutes of incubation is required for Ag-Ab reaction to take place in Normal Saline medium.
- **Centrifugation**- Centrifugation should be done at 1000 rpm for 1 min, over centrifugation may lead to formation of stronger cell button at the bottom of test tube which will be difficult to diffuse at the time of reading results.
- **Reagents**-Quality control of reagents and prevention of reagents from contamination during usage is very critical.
- **Technique of reading results**- After centrifugation the results should be read by diffusing the red cells button by gentle swirling of test tube. Any jerks or tapping may break the reactions and lead to downgrading of results or false negative results.
- **Addition of Coombs Control Cells**- Coombs control cells or IgG sensitized cells should be added in negative reactions for confirmation of negative results and validation of Coombs reagents.

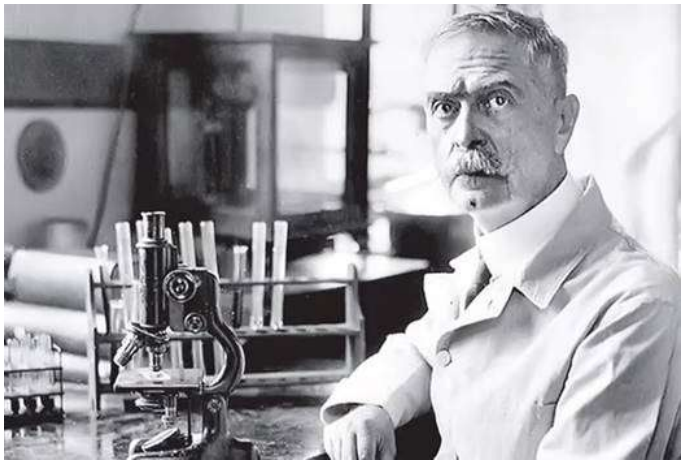
The above list of problems makes the Classical tube technique very complicated. The chances of errors are also very high. For a blood bank where many crossmatches are to be done in short period of time and a technician cannot process them in batches too, this forces them to make shortcuts to finish the test in time leading to the improper test results. Therefore most of the blood bank technicians perform only saline crossmatch that can be completed in 5-7 mins however this cannot detect the IgG class of antibodies which can cause the delayed transfusion reaction. Goes with the saying that ***"A test done wrong is worse than not done at all."***

Matrix™ Gel System:

Matrix™ Gel System offers solution to all these problems. In Matrix™ gel system washing step is eliminated. This saves a lot of time and eliminates the possibilities of error related to washing. The washing step is eliminated because of unique technology and procedural steps. Washing is done to remove the unreacted IgG antibodies and to prevent the neutralization of Coombs reagent, the same is achieved by following certain procedural steps in Matrix™ gel system. 0.8% Cell suspension is prepared by adding 10µl of packed cells in 1ml of Matrix™ Diluent -2 LISS. Usage of packed cells reduces the possibility of entry of IgG antibodies which are present in serum/plasma. The addition of 10µl packed cells to LISS without touching the LISS or walls of test tube also eliminates the entry of plasma and thus IgG antibodies. Out of this 1ml cell suspension, only 50µl is pipetted into the reaction chamber of gel card microtube. The addition of cell suspension and serum/plasma the following simple rules are to be followed:

- Always add cell suspension first.
- Cell suspension should be added at an angle of 45°.
- Serum/plasma should be added at an angle of 90° and through the wall of the microtube.

Addition of cell suspension and serum/plasma following the above rules facilitates the formation of air lock between mixture of cell suspension and serum/plasma. This air lock prevents the neutralization of Coombs reagent present in the gel microtube. Usage of Matrix™ Diluent-2 LISS enhances the reaction and therefore shortens the reaction time during incubation from 30-45 mins in classical tube technique to 15 mins in Matrix™ gel system. Normal saline is 0.98% Nacl which increases the electron cloud around the red cells and it takes longer time for antibody to reach and react with red cells. Matrix™ Diluent-2 LISS is Low ionic Strength Solution which contains potentiators. LISS reduces the electron cloud around the red cells and potentiators reduces the zeta potential and the desired reaction can be achieved in 15 mins.
Matrix™ gel system also offers stable end results, i.e. results can be read later and most important it helps user to standardize the procedures. The reaction read as 2+ by one technician can be read same by all the technicians. The results can be stored or documented by simply taking a Photo copy, scanning or even by taking a photograph of the card. Matrix™ Card Reader makes it simpler by offering automated reading and interpretation of results. These results can be stored in computer hard disc along with unique gel card barcode.



ANTIBODY SCREENING:

History of Blood Group Systems

Blood groups were discovered at the beginning of the twentieth century when Landsteiner noticed that plasma from some individuals agglutinated the red cells of others. As a result he succeeded in identifying the three blood groups A, B and O of human blood. With the development of the antiglobulin test by Coombs, Mourant, and Race in 1945, IgG class of antibodies could be detected and the science of blood group serology blossomed. This helped in discovery of different blood group systems. There are now about 270 authenticated blood group antigens. Many of these blood group antigens fall into one of 26 blood group systems listed in the below table.

ISBT No.	Name of Blood Group System	ISBT Symbol	No. of Antigens	Major Antigens
001	ABO	ABO	4	A, B, AB^1, A^1
002	MNS	MNS	43	M, N, S, s, U, Ena
003	P	P	1	P1
004	Rhesus	RH	46	D, C, E, c, e
005	Lutheran	LU	18	Lu^a, Lu^b, Lu^{ab}, Lu^4
006	Kell	KEL	24	K, k, Kp^a, Kp^b, Js^a
007	Lewis	LE	6	Le^a, Le^b, Le^{ab}
008	Duffy	FY	6	Fy^a, Fy^b, Fy3, Fy4
009	Kidd	JK	3	Jk^a, Jk^b, Jk^{ab}
010	Diego	DI	21	Di^a, Di^b, Wr^a, Wr^a
011	Cartwright (Yt)	YT	2	Yt^a, Yt^b
012	Xg	XG	2	Xg^a
013	Scianna	SC	3	Sm, Bu3, Sc3
014	Dombrock	DO	5	Do^a, Do^b, Gy^a, Hy, Jo^a
015	Colton	CO	3	Co^a, Co^b, Co^{ab}
016	Landsteiner-Wiener	LW	3	Lw^a, Lw^b, Lw^{ab}
017	Chido/Rodgers	CH/RG	9	Ch 1, Ch 2, Ch 3, Rg 1, Rg 2, WH
018	Hh	H	1	H
019	Kx	XK	1	Kx
020	Gerbich	GE	7	Ge 2, Ge 3, Ge 4, Wb
021	Cromer	CROM	10	Cr^a, Tc^a, Tc^b, Tc^c, Dr^a
022	Knops	KN	7	Kn^a, Kn^b, McC^a, Sl^a, Yk^a
023	Indian	IN	2	In^a, In^b
024	Ok	OK	1	Ok^a
025	Raph	RAPH	1	MER2
026	John Milton Hagen	JMH	1	JMH

Apart from these 26 Blood group systems there are few more antigens which are not assigned to any blood group system. Antibodies to all these antigens are not capable of causing Hemolytic Transfusion Reaction or HDFN. There are some antibodies which are clinically significant and capable of causing Hemolytic Transfusion Reaction and HDFN, such few antibodies are listed below.

Name of Blood Group System	Clinically Significant Antibodies
ABO	A & B
Rh	D, C, E, c, e
Kell	K
Kidd	Jk^a, Jk^b
Duffy	Fy^a, Fy^b
MNS	M, N, S, s
Lewis	Le^a, Le^b

Types of Antibodies

Expected or Natural Antibodies- If an ABO antigen is missing from an individual's red blood cell membrane, then it is **EXPECTED** that the individual will produce an antibody to that antigen. These have also been called **"naturally occurring"** antibodies. Example: Anti-A, Anti-B, Anti-AB.

Unexpected or Atypical Antibodies- In all other blood group systems if the antigen is missing from the red cell, the individual is **NOT** expected to produce an antibody against it, normally. When these antibodies are produced they are termed **UNEXPECTED or ATYPICAL or ALLOANTIBODIES**. The production of these antibodies is a result of an event like a transfusion or Pregnancy. These antibodies are of IgG class. Example: Anti-D, Anti-C, Anti Fy^a.

Pretransfusion Testing

The objective of pretransfusion testing is to ensure that enough red blood cells (RBCs) and components will survive when transfused, or in other words, to transfuse a blood component to a patient that will provide maximum benefit while causing less harm. Pretransfusion testing is very important, but the question is how to best and most economically do it?

In most blood banks, pretransfusion testing involves (i) determining the ABO and Rh types of patient and donor blood, (ii) screening patient and donor sera for RBC alloantibodies, and (iii) performing a major crossmatch (testing the patient's serum against the donor's RBCs). Pretransfusion testing can assure ABO compatibility between donor and patient blood as well as detect most clinically significant RBC alloantibodies that can react with donor RBC antigens.

Crossmatch or Compatibility testing

A major crossmatch, involves testing of the patient's serum with donor's RBCs, in IAT phase. Crossmatch should be performed before the transfusion of whole blood or RBC components. In crossmatch we detect the presence of antibodies in patient's serum/plasma corresponding to the antigens present on the donor's red cells.

"***A Negative or Compatible crossmatch shows that antibodies corresponding to the antigens of donor's red cells are absent in patient's serum/plasma. But a compatible crossmatch does not signify that there are no atypical antibodies present in patient's serum/plasma."***

Atypical antibodies are as you know are unexpected or alloantibodies.

Antibody Screening

Apart from clerical checking, grouping and typing of donor and patient blood, the serum or plasma of the patient must be tested against, single-donor, unpooled, group O reagent red cells. Such reagent red cells are selected because they carry the blood group antigens necessary for detecting the most important ***"clinically significant"*** RBC alloantibodies. This procedure is known as antibody screening and these reagent red cells are known as screening cell panels.

In transfusion service, a three cell panel for antibody-screening is routinely used to test for the presence of RBC alloantibodies. In brief, serum and reagent cells are incubated at 37^{O} C followed by indirect antiglobulin technique (IAT).

Antibody Screening is the most reliable and sensitive method of detecting alloantibodies. Crossmatch is often less reliable when compared with Antibody Screening, because some antibodies manifest dosage effect.

In order to understand the dosage effect we take an example of Kidd blood group system having two major antigens Jk^a and Jk^b. Please refer to below table

Cell	Jk^a	Jk^b	Remarks
Cell I	+	0	This cell is having Homozygous expression of Jk^a
Cell II	0	+	This cell is having Homozygous expression of Jk^b
Cell III	+	+	This cell is having Heterozygous expression of Jk^a and Jk^b

The above table explains the genotype for Jk^a and Jk^b, where '+' denotes the presence of antigen on the red blood cell and '0' denotes the absence.

In above example, cell I carries the Homozygous expression of Jk^a and results in higher expression of the Jk^a antigen than the cell III which carries the heterozygous expression of both Jk^a and Jk^b.

While performing crossmatch, phenotype (antigenic configuration of red cells) of donor's red cells is not known, and there are possibilities that, patient is having anti- Jk^a and donor red cell carries heterozygous expression of Jk^a. This may lead to a compatible crossmatch even in the presence of corresponding antibody. For these reasons, an antiglobulin crossmatch using donor cells is not the most effective way of detecting a serological incompatibility between donor and patient.

Antibody screening tests should detect all clinically significant antibodies Examples of such dosage-effect antibodies are anti-Jka, anti-Jkb, anti-Fya, anti-Fyb, anti-C, anti-E, and anti-c. Using three cell panel for antibody screening improves the chances of covering all major RBC antigens present in their homozygous forms during testing. In antibody screening is positive, further serological testing with an expanded panel (11 cell panel) of reagent RBCs for the identification of clinically significant antibodies is required. Then, once the specificity of the antibody is known, donor units must be screened for the corresponding antigen so as to select only those blood units which lack the antigen for transfusion.

According to NACO Guidelines published in 2007, in India, "Serum of the recipient should be tested for unexpected antibodies with screening red cell panel by indirect antiglobulin test with proper controls. If on screening, antibody/ies are detected, the antibody/ies should be identified by red cell panel, if possible".

For patient antibody screening we have Matrix™ ERYGEN –AS/ Reacell I, II, III three cell panel.

Donor Antibody Screening

The purpose of donor antibody screening is also to screen the donor's serum for the presence of alloantibodies. For donor antibody screening Pooled O cells may be used. This test should be done along with the grouping and typing of the donor's blood unit.

The AABB Standards also stipulate that blood from donors with a history of prior transfusion or pregnancy be tested for RBC alloantibodies, preferably at the time of processing. Most blood banks test all donor blood for RBC alloantibodies because of the difficulty in determining donors' past history. For this test, pooled reagent cells can be used to detect RBC alloantibodies in donor's blood.

According to NACO Guidelines published in 2007, in India, "Serum or plasma from donors should be tested for unexpected antibody/ies with pooled 'O' Rho D positive cells or preferably screening cell panel by indirect antiglobulin test which can identify clinically significant antibodies". Minor cross matching using donor's serum or plasma and recipient's cells should not be necessary as tests for complete and incomplete unexpected antibodies in donor samples are mandatory.

For donor antibody screening we have Matrix ERYGEN-PO, pooled O cells.

"Type and Screen" Policy

In some special instances, crossmatching of blood is excluded from pretransfusion testing according to a policy called "type and screen." This policy stipulates that blood does not have to be crossmatched in advance for patients undergoing surgical procedures usually not requiring blood. The patient's blood is, however, completely tested for ABO group, Rh type, and RBC alloantibodies and then kept in storage by the transfusion service in case it is needed for crossmatching. In most countries, typed and screened patient's blood can be crossmatched by IS-XM (immediate spin cross match or saline crossmatch) and made available in minutes, in India blood banks following Type and Screen policy perform Coombs crossmatch whenever blood unit is required for the patient.

"Type and Screen" can be very useful for the institutes having high crossmatch: transfusion (CT) ratio. CT ratio is the ratio of blood units crossmatched and blood units transfused in a hospital. Higher CT ratio suggested that number of blood units crossmatched is higher than the number of blood units transfused. Before the introduction of this procedure, many units of donor blood were crossmatched and held in reserve for patients who would probably not need it. At times this would cause shortages of the blood supply and unnecessary outdating of donor units. These factors, along with the added expense of crossmatching blood, caused the Type and Screen (T&S) procedure to

gain popularity.
This procedure is used most frequently to screen pre-operative or gynecological patients whose risk of excessive blood loss is minimal. In case of an emergency, where blood is needed for these patients, IS-XM (saline crossmatched), ABO and D compatible blood can be released with 99.9% assurance of safety, as long as the patient has no unexpected antibodies.

Antibody Screening along with concept of "Type and Screen" should be performed at all hospital blood banks having patients for routine surgery and gynaecology department.

Antenatal Antibody Screening- Hemolytic Disease of the Fetus and Newborn (HDFN)

The antigen that most frequently induces immunization is D, but, any red cell antigen present on fetal cells and absent from the mother can stimulate antibody production. As these antibodies are of IgG class, they are capable of crossing placenta and may cause hemolysis of fetal red blood cells. HDFN is often classified into three categories, on the basis of the specificity of the causative IgG antibody. In descending order of potential severity, they are:

1. D hemolytic disease caused by anti-D alone or, less often, in combination with anti-C or anti-E.
2. "Other" hemolytic disease caused by antibodies against other antigens in the Rh system or against antigens in other systems; anti-c and anti-K are most often implicated.
3. ABO HDFN caused by anti-A, B in a group O woman or by isolated anti-A or anti-B.

In order to detect these antibodies, the samples of all pregnant women should be taken early in pregnancy, ideally at 10-16 weeks gestation, for ABO and D typing and for screening for the presence of red cell alloantibodies. When an antibody screen is positive further tests should be carried out to determine the antibody specificity and significance.
All pregnant women, whether D positive or D negative, should have a further blood sample taken at 28 weeks gestation for re-checking the ABO and D group and further screening for red cell alloantibodies. D positive women are just as likely as D negative women to form antibodies, other than anti-D, late in pregnancy.
No further routine blood grouping or antibody screening is necessary after 28 weeks.
When red cell antibodies are detected, further testing of maternal blood should be undertaken to determine the specificity, concentration, origin and level of antibody or antibodies, and the likelihood of HDFN. Anti-D, anti-c and anti-K are the antibodies most often implicated in causing haemolytic disease severe enough to warrant antenatal intervention.

Antibody Screening along with concept of "Antenatal Antibody Screening" should be performed at all hospitals having gynaecology department.

Antibody Screening has got a wide application in terms of Immunohematology and Transfusion Medicine. However, sample found to be positive for antibody screening should be processed for antibody detection with 11 cell panel (Reacell 11 cell panel).

CASE STUDY AND PUBLISHED PAPERS WITH MATRIX GEL SYSTEM

Blood Group Discrepancy in a Donor Due to a Rare Ax/Aweak Subgroup Phenotype with *ABO*AW.31.01/*O.01.75* Genotype

Sneha Samir Babaria, Asha Purohit1, Vidhi S. Patel2

Introduction

The International Society of Blood Transfusion (ISBT) has identified 45 recognized human blood group systems with 360 red cell antigens by July 2023.[1] ABO antigens, crucial for transfusions and organ transplants, require accurate identification. A1 and A2 are common subgroups, distinguishable by anti-A1 lectin from Dolichosbiflorus.[2] Other rare variants such as A3, A intermediate (int.), Ax, Am, A bantu, A end, Ay, A finland (fin), A el, Ah (H-partially deficient, nonsecretor), and A weak, cause discrepancies in blood group testing.[3] Methods such as agglutination strength, ABO isoagglutinin presence, saliva secretor tests, and pedigree analysis help identify these variants.[4] Genotype *Aw06* has been previously reported in the Indian population. The *ABO*AW.31.01/*O.01.75* genotype was found in a 25-year-old male blood donor suspected of having an Ax/A weak blood phenotype.

Case Report

A 25-year-old male, belonging to the Rabari Community, migrated to Sanand city in Ahmedabad district, Gujarat, many years ago from Jaisalmer district, Rajasthan, and visited a tertiary care hospital in Western India as a blood donor. His weight was 98 kg and his hemoglobin level was 15.3 g/dL. The donor selection process followed WHO's technical guidelines. After collecting 450 mL of blood, a type II blood group discrepancy (weak ABO subgroups) was found using the tube technique as shown in Table 1.[5] Antisera used were monoclonal anti-A, anti-B, and anti-AB manufactured by Tulip Diagnostics (P) Ltd. Anti-D used were also monoclonal immunoglobulin (Ig) M plus IgG antibodies manufactured by Tulip Diagnostics (P) Ltd. and Diagast. To confirm the discrepancy, cell, and serum grouping was done with gel column agglutination technology (Matrix Octoplus Complete grouping Card, Gel system by Tulip Diagnostics (P) Ltd). The results were consistent with the tube technique observations [Figure 1].
Blood samples were sent to Tulip Diagnostics (P) Ltd. for further analysis. They conducted tests using their own gel card with different lot numbers, a gel card from Biorad, and tube testing with monoclonal anti-sera from Diagast and their own company, suggested an A2 (weaker variant) Rh-positive blood group. The results are shown in Table 1. Believing there might be another "A" subgroup besides "A2," the blood and saliva samples were sent to a higher blood bank center to verify the blood group. The referral center utilized reagent batteries, absorption-elution tests, and saliva analysis. Comments and conclusions are depicted in Table 2.

Table 1: Serological findings of the tube testing at the tertiary care center, and at the Tulip Diagnostics (P) Ltd. along with gel card method showing discrepancy

Antisera used	Blood grouping by different methods at various centers								
	Tube method at the tertiary care center								
	Forward grouping						Reverse grouping		
	Anti-A	Anti-B	Anti-AB	Anti-D	Anti-A_1	Anti-H	A cells	B cells	O cells
Tulip Diagnostics (P) Ltd.	0	0	2+	4+	0	4+	0	4+	0
Tube method at Tulip Diagnostics (P) Ltd.									
Tulip Diagnostics (P) Ltd.	0	0	0.5	3.5	0	4+	Not tested		
Diagast	0	0	Not tested	3	Not tested				
Gel card method at the Tulip Diagnostics (P) Ltd.									
Matrix octoplus	0	0	3+	4+	0	-	0	4+	0
BioRad	0	0	Not tested	3+	0	-	0	4+	Not tested

Table 2: Comments and conclusions of serological tests on the donor's blood and saliva by a higher blood bank centre

Comments:

Discrepancy in forward and reverse grouping showed the weak A phenotype No direct agglutination with anti-A but gave direct agglutination with anti-A, B reagents, albeit weaker than the normal complement of the antigen, in line with weaker variant of A antigen Absorption-elution experiment with positive elute further supported the presence of weak A on donor's red blood cells even though his RBCs failed to give direct agglutination with anti-A reagent Secretor status on his saliva with non-secretor phenotype is not of much help in the classification of the weaker variant found in the donor.

An impression of the Ax weaker variant category, further workup by testing blood and saliva of the family members and molecular aspects using DNA extract is needed. RBC: Red blood cells

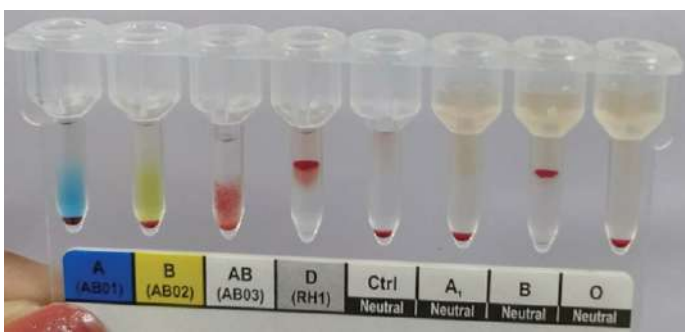


Figure 1: Gel card blood grouping at a tertiary care center (Matrix Gel system by Tulip Diagnostics (p) Ltd)

The higher center confirmed the Ax weaker variant suspected at the tertiary care hospital. To reach a final conclusion, family members' samples, and the donor's molecular test samples were requested but refused. Only the donor's blood sample was obtained for molecular study using next-generation sequencing through DNA extract.

Targeted sequencing utilized an Illumina DNA Prep with an enrichment kit plus a custom-designed panel which facilitates comprehensive genotyping for all blood group systems as per ISBT and transcription factors KLF1 and GATA1. The DNA sequence was aligned against the human reference (GRCh37) to identify variants relative to the reference sequence. Considering the predicted phenotype, Ax/Aweak, only the blood groups of interest in the sample were analyzed. Genetic variants found in the ABO gene were derived from changes observed between the sample and the Human Reference Genome (GRCh37) (GRCh37 allele: Exon 1–5: *ABO*O.01.02*, Exon: 6–7: *ABO*O.01.01*) and based on ISBT sequence transcript NM_020469.2. There were nucleotide substitutions consistent with heterozygosity for c.106T>G, c.188_189delATinsGC, c.220T>C, c.260_261insG and c.829G>A and homozygosity for c.297A>G, c.646T>A, c.681G>A, and c.771C>T and these variants define *ABO*AW.31.01* allele and Ax/Aweak phenotype. There was also nucleotide substitution c.542G>A, consistent with heterozygosity for c.542G>A, defining the *ABO*O.01.75* allele, however, one inconsistency was noted. This allele should also carry c.829G>A nucleotide substitution. While it was present in the sample, only at a heterozygous level linked to the *ABO*AW.31.01,* suggested that this change was expected to be present at a homozygous level. They have not confirmed whether c.829G>A was linked to the weak A allele or the O allele in the sample and made the assumption that it was linked to the A, without apparent effect on phenotype. Therefore, the predicted final genotype of the donor was *ABO*AW.31.01/*O.01.75.*

A blood donor card was given with a note to consider a person as "A" positive as a donor and as "O" positive as a recipient, along with advice to the donor not to donate blood in the future to prevent mistyping and ABO-incompatible transfusions to recipients who belong to the O Group.

Discussion

Transfusion of ABO-incompatible blood can cause severe hemolytic transfusion reactions, posing a higher risk than transfusion-transmitted diseases. ABO antigens are encoded by the ABO gene on chromosome 9, which has three main alleles: A, B, and O. Type II ABO discrepancy occurs due to reduced red cell antigen expression. There are more weak subgroups of A than B, and nearly 1% of ABO blood group variation is caused by weak A subgroups. The subgroup of A can be divided into two types based on their reaction with anti-A. Anti-A is agglutinated with A3, Aend, and Axe, but not with Ay, Ael, or Am. Thakral *et al.*'s research found that among weak subgroups of A, A3, and Axe have a population frequency of 1:14,448 in Indian blood donors. The most prevalent Ax allele possesses the A1 consensus sequence along with a missense mutation that codes for the replacement of Phe216Ile. Exons 6 and 7 mutations have often been tested for allelism, accounting for 77% of the ABO gene. However, weak subgroups are often mistyped as O group

due to variant techniques and reagents. Mistyped O red cell components may cause hemolysis when transfused to an O recipient. Proper identification of the ABO type is crucial for the effective management of "O" group RBCs and "AB" plasma in discrepant cases. Special tests such as serum glycosyltransferase and molecular testing help differentiate weak subgroups.

In this case study, the presence of a variant A allele was identified, with specific genetic changes defining the *ABO*AW.31.01* allele. This differs from research by Nair *et al*., who found the *Aw06/O13* genotype using polymerase chain reaction, and Seltsam *et al*., who reported the *Aw06* genotype in three patients with similar serological characteristics to subgroup Ax. The case displayed *ABO*O.01.75* allele with c.542G>A heterozygosity. Furthermore, c.829G>A substitution linked to *ABO*AW.31.01* showed no effect on phenotype. Studies suggest Ax phenotype inheritance varies with different modes and genetic backgrounds.

Conclusion

Weak subgroups of the ABO blood group system cause ABO discrepancies that are rarely observed in day-to-day activities. Due to identification errors, these groups might be mistakenly classified as O. If transfused to patients in the O Group, this could lead to a decrease in red cell survival because the recipient may naturally have anti-A antibodies, which could result in lethal transfusion reactions. Comprehensive serological testing and analysis, adsorption-elution, saliva secretor investigations, and molecular testing can help prevent discrepancies in blood typing. In this instance, the weak A phenotype with traits from serology *ABO*AW.31.01* and **O.01.75* was the ABO genotype that was comparable to Ax/A weak blood type. This is the first study to report the simultaneous changes in the A and O alleles in the Indian population, despite previous reports of the *Aw06* allele in the community. Molecular genetic methods aid in the accurate identification and description of weaker ABO subgroups and facilitate routine serology decision-making.

EXCERPTS FROM THE PUBLISHED PAPERS

Indian Journal of Pathology and Oncology 2023;10(1):34–39

Content available at: https://www.ipinnovative.com/open-access-journals
Journal homepage: www.ijpo.co.in
Original Research Article

A comparative study between gel card method and manual method for Coomb's test

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Conclusions

Our study showed that gel card is easier to use, more sensitive and less time-consuming with more standardized result and less sample needed for the test. Results of Gel card can be preserved for 3-4 days and this can be interpreted by various observers and compare it with the standardized grading system. Gel card assay appears to be an excellent method for detecting agglutination better than conventional tube method and easy to read weak agglutination and it can also detect ABO incompatibility. The performance of saline tube technique requires more experience and highly accuracy due to its long stages and multiple washing. But one disadvantage of gel card method is that gel cards are costly and require separate incubator and centrifuge.

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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).

Original Research Paper
VOLUME-6, ISSUE-11, NOVEMBER-2017 • ISSN No 2277 - 8160

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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.

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

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

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European Journal of Cardiovascular Medicine

Print ISSN: 2042-4884 | E-ISSN: 2042-4892 Origin: United Kingdom
Website: https://www.healthcare-bulletin.co.uk/ Volume 14 Issue: 2 - 2024

A comparative study to evaluate micro typing system gel card and conventional tube techniques for cross matching in a tertiary care Centre

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Conclusion

The basic principle of the gel test is that instead of a test tube, the serum and cell reaction takes place in a micro tube. Six of such micro tubes are embedded in a plastic card to allow ease of handling, testing, reading and disposal (21). Gel card Method is more sensitive and specific and better than conventional tube methods in terms of perseverance of result for 3-4 days, easy and quicker to perform, Person to person variation in results is absent and a less time consuming procedure, no cell washing is required and ideal for automation. Since gel has already been coated with AHG, there is no separate step of adding Ant globulin reagents. The only disadvantage of the gel card method is that gel cards are costly and need special enhancing mediums and instruments for this procedure. So, it is concluded that for routine compatibility testing in blood centres of all hospitals, gel card method is recommended.

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EVALUATION OF METHODOLOGY AND COMPARATIVE STUDY BETWEEN SPIN SALINE TUBE AND MATRIX GEL CARD TECHNIQUES FOR BLOOD COMPATIBILITY

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Conclusion

Matrix Gel card is more sensitive and more specific than Spin saline tube methods and also less time consuming but more costly than Spin saline tube methods. Matrix Gel Card technique is more stable and fully recordable for a long period. We can shoot the picture or scan of result and share or stored for further investigations. As per result, time consuming, recording, handling, less exposure, we concluded and advice for use of gel card in various blood banking services as routinely test performed in cross matching for blood transfusion because of high sensitivity and specificity then Spin saline tube methods. Matrix gel card method is better than Spin saline tube method because of its simplicity, stability of results, better handling, long time recorded, dispensation of controls with comparable sensitivity and specificity which is follow with this study. The result shows that gel test is more sensitive than tube test for identifying clinically potentiallysignificant of antibodies. Matrix gel card test also less time consuming than tube method with AHG reagent but cost effective method. We recommended that the usage of Matrix Gel card for routine blood cross-matching, blood grouping (forward and reverse) in all blood bank.

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The Efficiency and Specificity of Matrix Gel Method from the Forensic Point of View, in Determination of ABO Blood Grouping and Rhesus Factor

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Conclusion

From above observations it was concluded that the Matrix gel card technique is more suitable and less time consuming. The results are more stable can be recorded after long time.The test can be carried out with very small sample. In conclusion Matrix gel card test has been shown to be very efficient in forensic fields to get the blood group from the blood samples of Accuse, Victim, injured, Complainer Consisting of Male and Female.

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Journal of Allied Health Sciences
Gel Card and Saline Tube Techniques for Blood Cross-Matching: A Comparative Assessment Study

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Received date: February 5, 2022; Accepted date: May 11, 2022; Published date: August 31, 2022

Conclusion: Matrix gel card method is simple, easy to perform and gives more stable end point result that can be recorded and photocopied. It is more sensitive and specific than CTT. A larger cohort study is necessary to analyze the efficacy of Matrix gel card method over CTT without AHG.

CE Marking

The CE Mark is a symbol that signifies compliance with European Union (EU) regulations. The letters "CE" stand for "Conformité Européene," which translates to "European Conformity." The presence of the CE mark on a product indicates that it meets the stringent quality, performance, and safety standards set by the EU. Initially governed by Directive 98/79/EC, the CE mark is internationally recognized as a symbol of product reliability and compliance.

Compliance with Regulation (EU) 2017/746 (IVDR)

Regulation (EU) 2017/746, known as the In Vitro Diagnostic Regulation (IVDR), replaces Directive 98/79/EC and establishes a comprehensive legal framework for in vitro diagnostic (IVD) medical devices in the EU. It aims to ensure high safety and performance standards while supporting innovation and the seamless movement of these devices within the European market. The regulation became fully applicable on May 26, 2022.

Key aspects of Regulation (EU) 2017/746:

- Strengthens oversight of IVD devices to ensure safety and effectiveness.
- Introduces a risk-based classification system (Class A to D).
- Requires manufacturers to conduct performance evaluations and provide clinical evidence.
- High-risk devices (Class D) must be certified by a notified body.
- Implements a Unique Device Identification (UDI) system for better traceability.
- Requires continuous monitoring and reporting of device performance and safety issues.
- Establishes the European Database on Medical Devices (Eudamed) to centralize information.
- Mandates clear labeling and public access to essential safety and performance data.
- Reinforces requirements for genetic testing and patient counseling.
- Defines responsibilities for manufacturers, importers, and distributors.
- Requires non-EU manufacturers to appoint an EU representative.
- Enhances oversight of notified bodies assessing compliance.
- Establishes EU reference laboratories for high-risk IVDs.

CE Certification Process for In-Vitro Diagnostic Devices

To market in vitro diagnostic devices in the EU, manufacturers must meet strict quality requirements and obtain CE certification. The process involves:

1. **Quality Assurance Certification:**
   Manufacturers must first obtain the following certifications:
   - ISO 9001:2000
   - ISO 13485
2. **Conformity Assessment Procedures:**
   The manufacturer must follow specific conformity assessment procedures outlined in the regulation. These procedures vary based on the device's risk classification.
3. **Notified Body Involvement:**
   Manufacturers must apply to a designated notified body for conformity assessment. The notified body evaluates documentation, verifies compliance with technical standards, and ensures product safety and performance. For high-risk Class D devices, additional laboratory testing by an EU reference laboratory is required.
4. **Post-Market Surveillance and Compliance:**
   - Notified bodies conduct periodic audits, unannounced inspections, and sample testing.
   - Manufacturers must monitor and report performance issues or adverse effects.
   - Continuous compliance ensures the device meets regulatory requirements for safety and performance.

CE Certification for Blood Grouping Reagents

Blood grouping reagents fall under Class D (previously Annex II List A under Directive 98/79/EC) due to their high-risk nature. The certification process for these reagents follows strict oversight, including:

1. **Conformity Assessment Options for Class D Devices:**
   - **Annex IX:** Comprehensive quality management system assessment and technical documentation review.
   - **Annex X & XI:** Type examination (Annex X) combined with production quality assurance (Annex XI).
2. **Notified Bodies and EU Reference Laboratories:**
   - A notified body conducts conformity assessments to verify compliance.
   - The EU Reference Laboratory (EURL) verifies claimed performance through laboratory testing, focusing on analytical and diagnostic sensitivity.
3. **Additional Scrutiny for Class D Devices:**
   - Notified bodies must inform competent authorities when issuing a certificate for a Class D device.
   - The EURL's scientific opinion is required for batch verification.
   - A negative opinion from the EURL prevents certification.
4. **Batch Verification Requirements:**
   - Each batch undergoes manufacturer testing before submission to the notified body.
   - Samples may be sent to the EURL for independent testing.
   - If the notified body does not raise objections within 30 days, the device can be marketed.

This rigorous assessment process ensures that Class D devices meet high safety and performance standards before reaching the market.

Performance Evaluation of Tulip's Blood Grouping Reagents

Tulip's blood grouping reagents have successfully undergone conformity assessments as per the **Commission Decision of November 27, 2009, amending Decision 2002/364/EC** on common technical specifications for in vitro diagnostic medical devices and presumed to be in conformity with performance requirements set out in section 9.1 (point a and b), section 9.3, and section 9.4 of Annexure I to Regulation 2017/746.

Study Details:

- **Study Period:** December 22, 2017 – July 30, 2018
- **Location:** Tulip Diagnostics (P) Ltd, Verna, Goa
- **Sample Size:**
  - 2709 blood donors
  - 403 clinical samples
  - 95 newborn samples
  - 68 D weak samples

The ABO blood group distribution met regulatory requirements:

- A: 24.7%
- B: 26.2%
- AB: 9.4%
- O: 39.8%

For the Rh system, the distribution was:

- Rh-positive: 91%
- Rh-negative: 9%

Results from 3,269 slide tests and 3,275 tube tests showed excellent correlation with CE-marked reagents. No discrepancies were observed in test results for **Eryclone Anti-A, Eryclone Anti-B, Eryclone Anti-A,B, Eryclone Anti-D (IgM), and Rhofinal Anti-D (IgM+IgG)**. Weak D samples were confirmed using Indirect Antiglobulin Testing (IAT).

Conclusion

The performance evaluation of **Eryclone Anti-A, Eryclone Anti-B, and Eryclone Anti-A,B** on over 3,275 samples demonstrated 100% Specificity and Sensitivity across common phenotypes (A1, A2, A1B, A2B, B, and O).

The performance of **Eryclone Anti-D(Rho)(IgM)** was evaluated on over 3275 samples (from donors, patients and neonates) drawn in the recommended anticoagulants. The evaluation demonstrated 100% specificity.

The sensitivity of the reagent by slide test is 98.77% and by tube test it is 99.37% versus the expected results with commonly known Rhesus phenotypes.

The performance of **Rhofinal Anti-D(Rho)(IgM+IgG)** was evaluated on over 3275 samples (from donors, patients and neonates) drawn in the recommended anticoagulants. The evaluation demonstrated 100% specificity. The sensitivity of the reagent by slide test is 99% and by tube test it is 99.5% versus the expected results with common known Rhesus phenotypes.

Performance Evaluation of Tulip's Matrix Gel Cards

Tulip's Matrix Gel Cards have successfully undergone conformity assessments as per the **Commission Decision of November 27, 2009, amending Decision 2002/364/EC** on common technical specifications for in vitro diagnostic medical devices and presumed to be in conformity with performance requirements set out in section 9.1 (point a and b), section 9.3, and section 9.4 of Annexure I to Regulation 2017/746.

Study Details:

- **Study Period:** December 14, 2017 – July 30, 2018
- **Location:** Tulip Diagnostics (P) Ltd, Verna, Goa
- **Sample Size:**
  - 2709 blood donors
  - 403 clinical samples
  - 95 newborn samples
  - 68 D weak samples
  - 17 clinical samples with atypical antibody

Conclusion

The performance evaluation of **Matrix Gel Cards** on over 3,292 samples shows excellent correlation with the results obtained with the CE marked commercially available reference reagent and/or cards.

Tulip's blood grouping reagents and Matrix Gel Card are **CE certified** under:

- **EC Certificate No. 1434-IVDD-144/2022**
- **EC Certificate No. 1434-IVDD-145/2022**
- **EC Certificate No. 1434-IVDD-146/2022**

Certified as per **Directive 98/79/EC**, valid until **December 2027** under **Regulation (EU) 2017/746**.

Tulip's Blood Grouping reagents and Matrix Gel Cards uphold the highest international quality standards.

BOUQUET

In Lighter Vein

When I was a kid, My English teacher looked my way and said, "Name two PRONOUNS"

I said "WHO ME?""

Teacher: Can a kangaroo jump higher than Eiffel Tower?

Student: Yes Sir.

Teacher: Why?

Student: Because the Eiffel tower can't jump!

Son: Dad ! I'm going out…!

Dad: are you telling me or Asking me Permission?

Son: I am asking you for money…!

A son asked his Mom about his Birthday Gift…..

Naughty Boy: Mummy, What will I get for my birthday?

Mummy: Close your eyes. Now tell me what you see?

Naughty Boy: Nothing. Mummy.

Mummy: That's what…

Wisdom Whispers

"The First Step to getting anywhere is deciding you are no longer willing to stay where you are."

"Life always offers you a second chance…It is called TOMORROW."

"Be Yourself, Everyone Else is already taken."

"Your problem isn't the problem. Your reaction is the problem."

Brain Teasers

1. **Gel technology in blood banks is also known as____**
   a) Column agglutination technology
   b) Tube centrifugation technology
   c) Micro agglutination technology
   d) Coombs test

2. **_____ was designed and developed by Dr. Yves Lapierre of France**
   a. Blood Group
   b. Antibody screening
   c. Gel technology
   d. Micro agglutination technology

3. **Red cell suspension is prepared in LISS _____**
   a. Reduces electron cloud around the red cells
   b. Reduces Zeta potential
   c. Strong agglutination & Reduces incubation time
   d. All of the above

4. **Antibody screening & Identification is done to detect _____.**
   a. Expected antibodies
   b. Weaker groups
   c. Unexpected antibodies
   d. None of the above

ANSWER: 1:a, 2:c, 3:d, 4:c.

Automation for Every Blood Bank

Introducing

matrix™ GEL•SYSTEM **AutoMini 40**

The STAR

Simplistic Design
Technological Excellence
Agile Performance
Reliable Results

- *Fully Automated Processor for blood grouping, cross matching and antibody screening...*
- *Modular System with STAT facility*
- *Single needle system for preparing cell suspension*
- *Integrated Barcode System for Samples & Reagents*
- *Automatic Clot, Liquid level detection*
- *Bidirectional LIS facility*
- *Total Security & Integrity*

Matrix™ Gel System - Card Parameters

- **Matrix™** Forward & Reverse Grouping Card with Autocontrol
- **Matrix™** Forward Grouping Confirmation Card
- **Matrix™** Forward Grouping DAT Card
- **Matrix™** Neonate Group Card
- **Matrix™** AHG (Coombs) Test Card
- **Matrix™** Coombs Anti-IgG Card
- **Matrix™** Forward Grouping Crossmatch card
- **Matrix™** Neutral Gel Card
- **Matrix™** Coombs and Neutral Gel Card
- **Matrix™** Rh Phenotype Card with Anti-D
- **Matrix™** Rh Phenotype Card with Anti-K
- **Matrix™** ABO Subgrouping Card Anti-A1
- **Matrix™** ABO Subgrouping Card Anti-H
- **Matrix™** Direct Antiglobulin Test Card

Printed and published by D.G. Tripathi, Edited by Dr. Ramnik Sood, M.D. (Path.) for and on behalf of Tulip Diagnostics Private Ltd., Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex Post Office, Goa - 403 202, INDIA.
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