NIB/BRL/GM/02

Guidance manual on
"ABO and Rh blood grouping"

NATIONAL INSTITUTE OF BIOLOGICALS
(Ministry of Health & Family Welfare)
Government of India
A-32, Sector -62, Institutional Area
NOIDA- 201307

NIB, BLOOD REAGENT LAB

AMENDMENT SHEET

S.NO	Date	Page no.	Revision No.	Nature of Amendment Section/ details	Authorization

FOREWORD

Blood transfusion is an essential part of modern health care system and if blood with correct group is transfused it can save precious life. Blood grouping plays an essential part of Blood transfusion service.

Blood Reagent Laboratory at National Institute of Biologicals, Noida is the only Central Drugs Laboratory for *in-vitro* Blood Grouping Reagents and has been evaluating the reagents forwarded by DCG (I), since 1997.

The Guidance Manual on "ABO and Rh grouping" describes the methodology used for Grouping, sub-grouping, Rh phenotyping of blood for determination of human blood group before transfusion. The purpose of preparation of this Guidance Manual is to strengthen the Blood transfusion services by correct grouping of red blood cells and plasma which can save precious lives and improve health.

ABBREVIATIONS USED

BGR	Blood Grouping Reagent
BRL	Blood Reagent Laboratory
BSA	Bovine Serum Albumin
DCG(I)	Drugs Controller General of India
HDN	Hemolytic Disease of the New Born
HTR	Hemolytic Transfusion Reaction
IS	Immediate Spin
IAT	Indirect Agglutination Test
IRCS	Indian Red Cross Society
IP	Indian Pharmacopoeia
IPC	Indian Pharmacopoeia Commission
NABL	National Accreditation Board for Testing and Calibration Laboratories
NS	Normal Saline
NIB	National Institute of Biologicals
NIBSC	National Institute of Biological Standards and Control
PBS	Phosphate Buffered Saline
QA	Quality Assurance
QC	Quality Control
RBC	Red Blood Cell
RT	Room Temperature
SOP	Standard Operating Procedure
TTI	Transfusion Transmitted Infection

CONTRIBUTORS

BLOOD REAGENT LABORATORY

Dr. J.P.Prasad	Scientist Grade II Laboratory Head	jpprasad@nib.gov.in
Mrs. Kanchan Ahuja	Scientist Grade III Laboratory In-charge	kannib69@gmail.com
Dr. Meena Kumari	Junior Scientist	drmeena.7@gmail.com
Ms. Priya	Laboratory Assistant	mailpriya.bhatt75@gmail.com

ACKNOWLEDGEMENT

I would take this opportunity to express my gratitude to all the scientific staff of Blood Reagent Laboratory- Ms. Kanchan Ahuja, Dr. Meena Kumari and Ms. Priya Bhatt for providing the valuable inputs in the preparation of the Guidance Manual on "ABO and Rh blood grouping" without which the endeavor would not have been possible. I also wish them good luck in their future scientific work.

The encouragement provided by Dr. Surinder Singh- Director (I/c) to prepare this document is deeply acknowledged.

Last but not least, I sincerely acknowledge the help rendered by Indian Red Cross Society, New Delhi and Dr. Vanashree Singh, Director, IRCS for providing valuable material for the preparation red cell panel.

I hope the Guidance Manual on "ABO and Rh blood grouping" will go a long way in Blood Transfusion Services.

Dr. J.P.Prasad
Laboratory Head
Blood Reagent Laboratory

PURPOSE

The purpose of this guidance manual is to assist Blood Transfusion Services - Blood banks, hospitals to strengthen ABO and Rh blood grouping methodologies which are routinely used in the blood banks.

SCOPE

This guidance manual provides general information on procedures and practices involved in blood grouping and may be useful to Blood Transfusion Services- blood banks, hospitals. Transfusion services may follow these guidelines or may choose to use alternative procedures not provided in the guidelines, however these alternative procedures must be established by International or National Regulatory Authority.

The methodology, specifications, and other matters referred in this manual are intended to assist Blood transfusion services in improvement of procedures used in Blood grouping.

INTRODUCTION

National Institute of Biologicals is an autonomous institute under the Ministry of Health & Family Welfare set for the quality assessment of biological products manufactured indigenously and imported in the country, works in coordination with the Regulatory Authorities such as Drugs Controller General of India DCG (I) and the Indian Pharmacopoeia Commission.

The Blood Reagent Laboratory is a notified laboratory by Government of India vide Gazette No. 158, dated 4th April, 2002 for quality control evaluation of Blood Grouping Reagents. The laboratory is also NABL accredited for Biological and Chemical tests as per ISO 17025:2005. Since 1997 the laboratory has evaluated a total number of 1313 batches of routine and rare blood grouping reagents. The laboratory has a repository more than 50 cryopreserved panel members for routine and rare red blood cells.

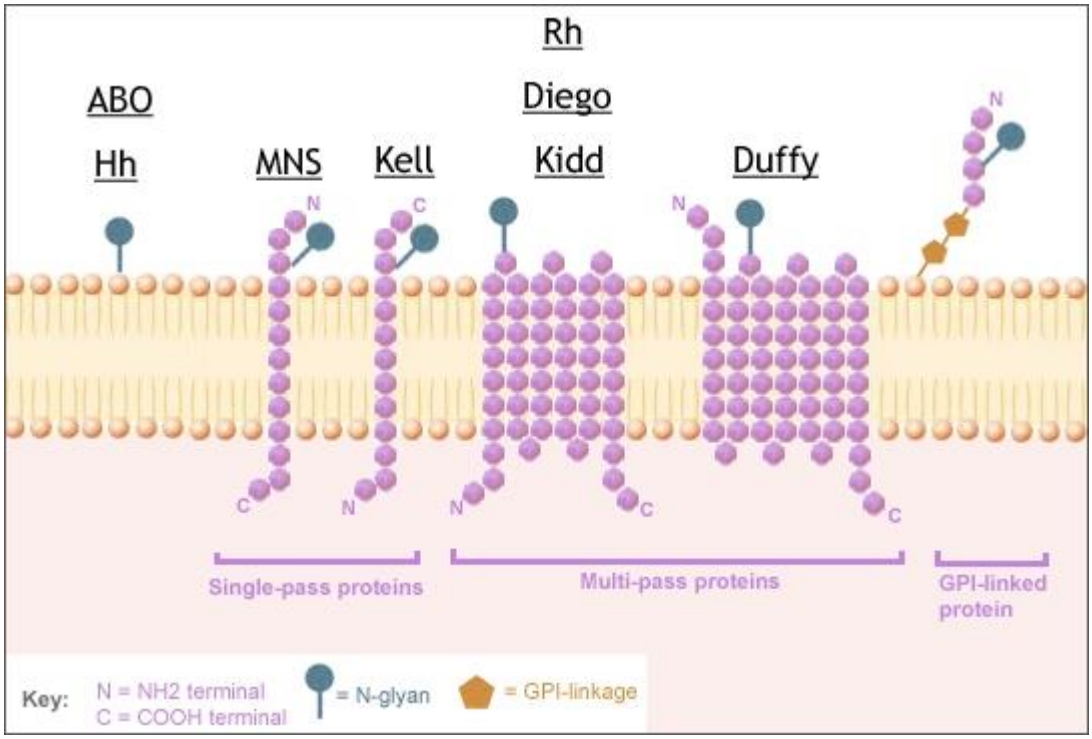
In view to improve the Blood Grouping techniques, the 1st edition of Guidance Manual on "ABO and Rh blood grouping" is developed by National Institute of Biologicals which can be used by the Blood Transfusion Services to improve their procedures.

The recommended methods are provided to help the user to have consistent and reliable products in conformity with specific standards and help to improve the quality of Blood transfusion Services. To ensure the continued safety of the nation's blood supply, it is essential that Blood transfusion Services implement effective methodologies for Blood Grouping.

I ABO GROUPING

The cells that make up the body's tissues and organs are covered with surface markers, or antigens. Blood group antigens are either sugars or proteins, and they are attached to various components in the red blood cell membrane. The ABO blood group are sugars (glycan or carbohydrate).

The figure below shows the red blood cell membrane and some of the blood group antigens attached to it. The red blood cell membrane contains three types of protein: single-pass proteins, multi-pass proteins, and glycosylphosphatidylinositol- linked proteins.



Blood groups differ around the world

The distribution of the four ABO blood types, A, B, AB, and O, varies in populations throughout the world. It is determined by the frequency of the three alleles of the ABO gene in different populations. Blood type O is the most common worldwide, followed by group A. Group B is less common, and group AB is the least common.

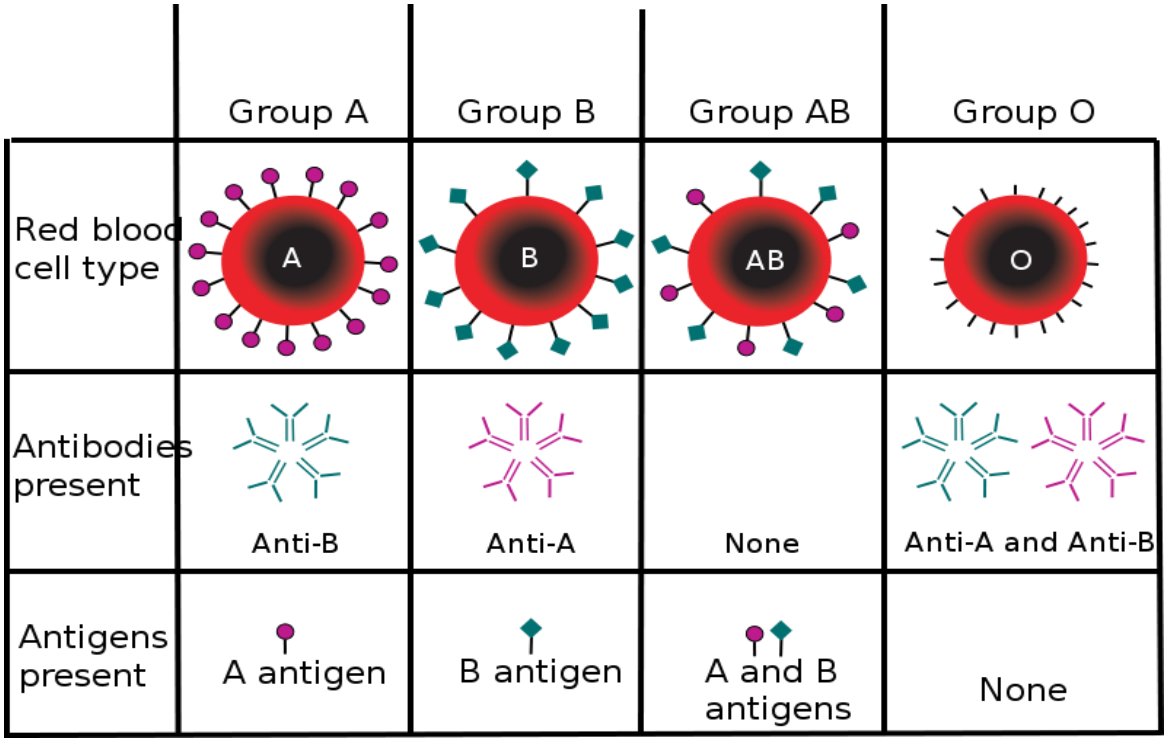
Blood groups

A blood group system contains antigens controlled by a single gene. There are 22 blood group systems, including the ABO, Rh, and Kell blood groups which contain antigens that can provoke the most severe transfusion reactions.

Each blood group antigen is assigned a six-digit number by the ISBT. The first three digits represent the blood group (e.g., ABO is 001, Rh is 004), and the last three identify the antigen in the order it was discovered. For example, for ABO, the A antigen was the first to be discovered and has the number 001.001 whereas the B antigen was next and is designated 001.002.

ABO Blood Type	**Per Cent of General Population**	**Chance of Finding A Compatible Donor**
O+	**38.5%**	1 out of 2 -50%
O-	6.5%	1 out of 15-7%
A+	**34.3%**	4 out of 5- 80%
A-	5.7%	1 out of 8- 13%
B+	**8.6%**	3 out of 5- 60%
B-	1.7%	1 out of 12- 9%
AB+	4.3%	**100%**
AB-	0.7%	1 out of 7- 14%

ABO ANTIGEN- ANTIBODY SUMMARY CHART



II The Rh blood group

The Rh blood group is one of the most complex blood groups known in humans. From its discovery 60 years ago where it was named (in error) after the Rhesus monkey, it has become second in importance only to the ABO blood group in the field of transfusion medicine.

The antigens of the Rh blood group are proteins. A person's DNA holds the information for producing the protein antigens. The RhD gene encodes the D antigen, which is a large protein on the red blood cell membrane. Some people have a version of the gene that does not produce D antigen, and therefore the RhD protein is absent from their red blood cells. To date, 49 Rh antigens are known.

The significance of the Rh blood group is related to the fact that the Rh antigens are highly immunogenic. In the case of the D antigen, individuals who do not produce the D antigen will

produce anti-D if they encounter the D antigen on transfused RBCs (causing HTR) or on fetal RBCs (causing HDN). For this reason, the Rh status is routinely determined in blood donors, transfusion recipients, and in mothers-to-be.

Antigens of the Rh blood group

Number of antigens	**49: D, C, E, c, and e are among the most significant**
Antigen specificity	**Protein** The sequence of amino acids determines the specificity of most of the Rh antigens.
Antigen-carrying molecules	**Proteins with unknown function** The RhD and RhCE proteins are both transmembrane, multipass proteins that are integral to the RBC membrane.
Molecular basis	**Two genes, RHD and RHCE, encode the Rh antigens.** The Rh genes are 97% identical, and they are located next to each other on chromosome 1.
Frequency of Rh antigens	**D**: 85% Caucasians, 92% Blacks, 99% Asians **C**: 68% Caucasians, 27% Blacks, 93% Asians **E**: 29% Caucasians, 22% Blacks, 39% Asians **c**: 80% Caucasians, 96% Blacks, 47% Asians **e**: 98% Caucasians, 98% Blacks, 96% Asians (1)
Frequency of Rh phenotypes	**Rh haplotype DCe**: most common in Caucasians (42%), Native Americans (44%), and Asians (70%) **Rh haplotype Dce**: most common in Blacks (44%) **Rh D-negative phenotype**: most common in Caucasians (15%), less common in Blacks (8%), and rare in Asians (1%) (1)

Antibodies produced against Rh antigens

Antibody type	**Mainly IgG, some IgM** The majority of Rh antibodies are of the IgG type.
Antibody reactivity	**Capable of hemolysis** Rh antibodies rarely activate complement. They bind to RBCs and mark them up for destruction in the spleen (extravascular hemolysis).
Transfusion reaction	**Yes—typically delayed hemolytic transfusion reactions** Anti-D, anti-C, anti-e, and anti-c can cause severe hemolytic transfusion reactions. Hemolysis is typically extravascular.
Hemolytic disease of the newborn	**Yes—the most common cause of HDN**. Anti-D and anti-c can cause severe disease. Anti-C, anti-E, and anti-e can cause mild to moderate disease.

Nomenclature

- Number of Rh antigens: 49
- ISBT symbol: Rh
- ISBT number: 004
- Gene symbols: RHD and RHCE
- Gene names: Rhesus blood group, D antigen; and, Rhesus blood group, CcEe antigens

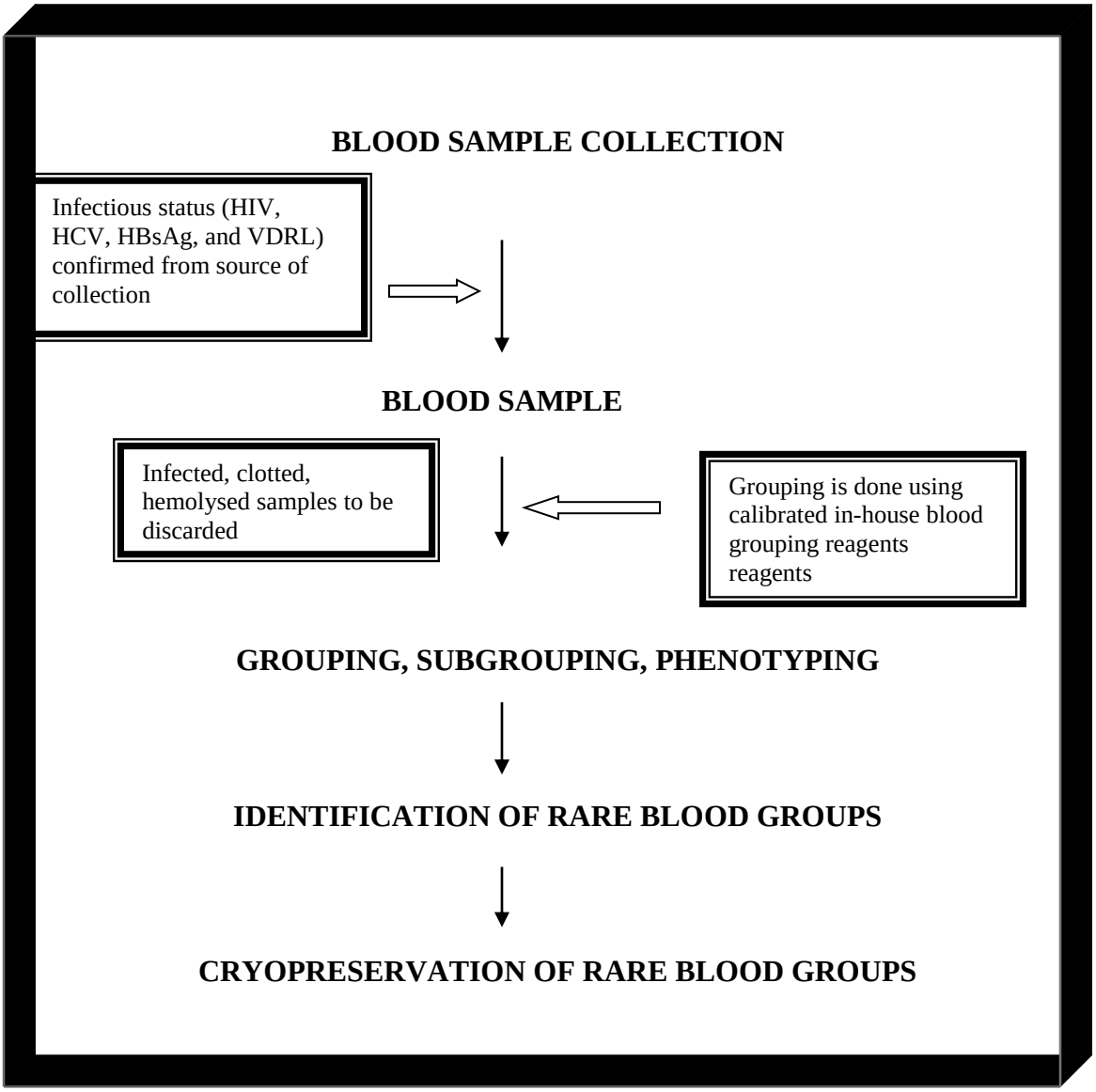
The D antigen contains over 30 epitopes. Variations of the D phenotype arise when these epitopes are only weakly expressed ("weak D phenotype") or when some are missing ("partial D phenotype").

Weak D: all D antigen epitopes are present but are under expressed

"Weak D" is a Rh phenotype found in less than 1% of Caucasians and is only slightly more common in African Americans. It is typically caused by a single amino acid switch in the transmembrane region of the RhD protein. hemolytic transfusion reaction, HTR. Therefore, individuals with the weak D phenotype can receive Rh D-positive blood.

The Rh locus is located on the long arm of chromosome 1. It contains the RHD and RHCEgenes, which lie in tandem.

FLOW CHART FOR SAMPLE PROCESSING



A BLOOD SAMPLE COLLECTION

Sample Collection

- Collect sample in a screw cap sterile vial with the help of Blood Bank Medical / technical staff on duty.
- Label source, sample number, date and blood group of the donor on sample vial.
- Note down sample details in the notebook.
- Cross check sample details marked on vials with those recorded in note book.
- Tighten vial screw cap & keep in icebox with ice pack (at 2-8^{0}C).
- **Confirm TTIs testing results for collected samples.**
- Destroy and discard the used gloves.

3. Biosafety Aspects

All blood samples should be considered potentially infectious hence all biosafety Precautions should be followed as discussed in SOP for Biosafety.

- Discard all washing supernatant into 1% sodium hypochlorite.
- Ensure that used tips, vials are properly soaked in 1% sodium hypochlorite
- Confirm results for TTIs and document in register (Blood sample collection) and discard as per SOP if found reactive for any TTI.

4. Sample storage & documentation

- Label BRL Sample No. on respective sample vials and store in dedicated refrigerator.
- Document sample details in sample collection register
- Confirm TTI result for collected samples from source as soon as possible and record in respective register
- Disinfect & discard the sample if
  - ✓ Vial is broken
  - ✓ Reactive for HCV / HIV / HBV/VDRL
  - ✓ Haemolysed / clotted
  - ✓ Period from date of collection exceeds 35 days.

B GROUPING AND SUBGROUPING

Three manual methods can be used when performing blood grouping:

- Glass slide or white porcelain tile
- Glass test tube
- Microwell plate or microplate

Newer techniques
- Column technique (sephadex gel)
- Solid phase tests

1) Slide or Tile Method

This technique may be used for emergency ABO grouping tests or for preliminary grouping particularly in an outdoor camp.
Slide or tile testing is not recommended for routine use because it is not reliable for

- weakly reactive antigens on cells
- serum grouping with low titre anti-A or anti-B

Disadvantages

- Less sensitive than the tube test
- Drying up of the reaction mixture can cause aggregation of cells , giving false positive results
- Weaker reactions are difficult to interpret.

2) Microplate Technique

Microwell plate consists of a small tray with 96 small wells each of which can hold about 200-300u1 of reagent. Microplate technology is gaining widespread popularity due to increasing workload in blood transfusion laboratories and recent availability of packaged automated system.

Three types of microplates are available
a. U-type well
b. V-type well
c. Flat-bottom

The U-type well is generally used in red cell serological work as it is easier to read the results in U- bottom plates.

Advantages of Microplate ABO grouping

1. Small volumes and low concentration of sera and red cells are used, making it cost-effective.
2. Easy handling of a microplate, which can replace 96 test tubes.
3. Batching of samples can be achieved with considerable economy in space and time.
4. If larger laboratories acquire microplate hardware items e.g. reagent dispenser, sample handler and cell washer it may further reduce the operation time.
5. Large batches of plates can be predispensed with antisera and reagent red cells before testing.
6. The technique of microplate grouping may be automated by on-line data capture in larger laboratories, which may help in
   a) reduction in reading and transcription errors
   b) saving in staff time
   c) use of bar codes for samples and microplate identification
   d) integration into a comprehensive computer system for storage of data.

3) <u>Tube Method</u>

Test tubes either of glass or plastic may be used. The tube technique is more sensitive than slide technique for ABO grouping.

Advantages of tube method

- It allows for fairly long incubation without drying up of the tubes contents.
- Centrifugation involved enhances the reaction allowing weaker antigens and antibodies to be detected.
- Simplicity of reading and grading of results.
- Clean and more hygienic.
- Requires smaller volume of reagents
- More sensitive than slide technique

GROUPING BY TUBE METHOD

Samples

CPDA anti coagulated Blood samples collected from any source.

Reagents required for tube method

- Working standardised Monoclonal antisera (Anti-A, Anti-B, Anti-A,B)
- Anti – A_1 (Lectin) & Anti - H (Lectin)
- Reagent cells (A cells, B cells and O cells), 3% in Normal Saline.
- Test red cells – Samples from IRCS
- Normal Saline (0.9%)

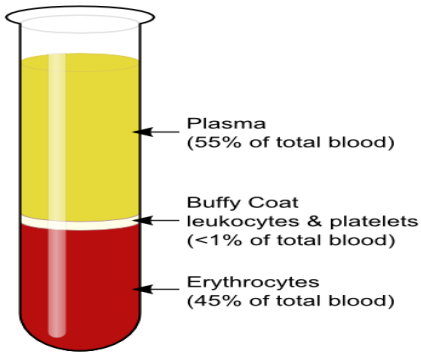
Bench Preparation (NIB/BRL/SOP/44/R1)

- Allow all reagents to come to Room Temperature
- Identify reagents RBC / blood samples to be used for reverse grouping
- Fill in proforma

Processing of blood samples

Separation of RBC & Plasma

Centrifuge at 1000 rpm for 1 min at R.T using clean pipette tip, aspirate plasma gently without disturbing settled cells and transfer to a labeled clean test tube for **reverse grouping**.



Preparation of cell Suspension (NIB/BRL/SOP/36,40,41/R1)

- Label the tubes as per S.No. of samples
- Add 1ml whole blood in respective S.No. of tubes and Normal saline (N.S) 8 ml, mix well.
- Centrifuge at 2500 rpm for 3 min at R.T
- Aspirate supernatant & discard
- Wash 3 times as above till supernatant is clear.
- Consider cell pellet as 100%
- Prepare 3%red cells suspension in normal saline

To prepare 100ul of required %suspension, mix N.S & packed RBC as below;

Preparation of % RBC suspension

% of cells	Vol. of N. S (µl)	Vol. of Washed, packed RBC (µl)
1%	**99**	**1**
2%	**98**	**2**
3%	**97**	**3**
5%	**95**	**5**
40%	**60**	**40**

Setting up tubes

Set 9 tubes for each test sample as follows;

- 3 tubes labelled - A, B, A B. (forward/cell grouping)
- 2 tubes labelled - H & A_1
- 3 tubes labelled Ac, Bc and Oc (reverse/serum grouping)
- 1 Auto control tube, Add 100ul test serum and 50ul test cells suspension of same sample and label it.

I <u>Forward grouping (cell grouping)</u>

- Add 100ul each of Anti-A, Anti-B, Anti-AB, Anti-A_1,and Anti-H in respective labelled tube.
- Add 100ul of 3%test cell suspension in the five tubes labelled A, B, AB, A_1 & H.

II <u>Reverse grouping (serum grouping)</u>

- Add 100ul each of the test serum in tubes labelled Ac, Bc and Oc.
- Add 50ul each of reagent A cells, B cells and O cells in the above tubes respectively.
- Mix the contents of all the 9 tubes by shaking the tube rack carefully and centrifuge at 1000 rpm for 1 minute.
- Dislodge cell button by gently shaking the tubes and read against well-lit background
- Grade and record agglutination reactions.

INTERPRETATION OF RESULTS

Grouping

Cell grouping			Serum grouping			Result
Anti-A	Anti-B	Anti-AB	Ac	Bc	Oc	
+	-	+	-	+	-	A
-	+	+	+	-	-	B
-	-	-	+	+	-	O
+	+	+	-	-	-	AB
-	-	-	+	+	+	Oh or any other irregular antibody

III Sub-grouping

Group	**A_1 (Lectin)**		**Anti – H (Lectin)**
	A_1/ A_1B	**A_2/A_2B**	
A	**+**	**-**	**Should give intensity of reaction in order**
AB	**+**	**-**	
B	**NA**		**$O>A_2>A_2B>B>A_1>A_1B$**
O			
O^h Bombay Group			**Neg**

Grading of Agglutination

Defining the Strength of Reaction (Grading of Agglutination)

To record the difference in the strength of reaction, it is necessary to have a

system of grading or scoring the reactions, as depicted in figure-1

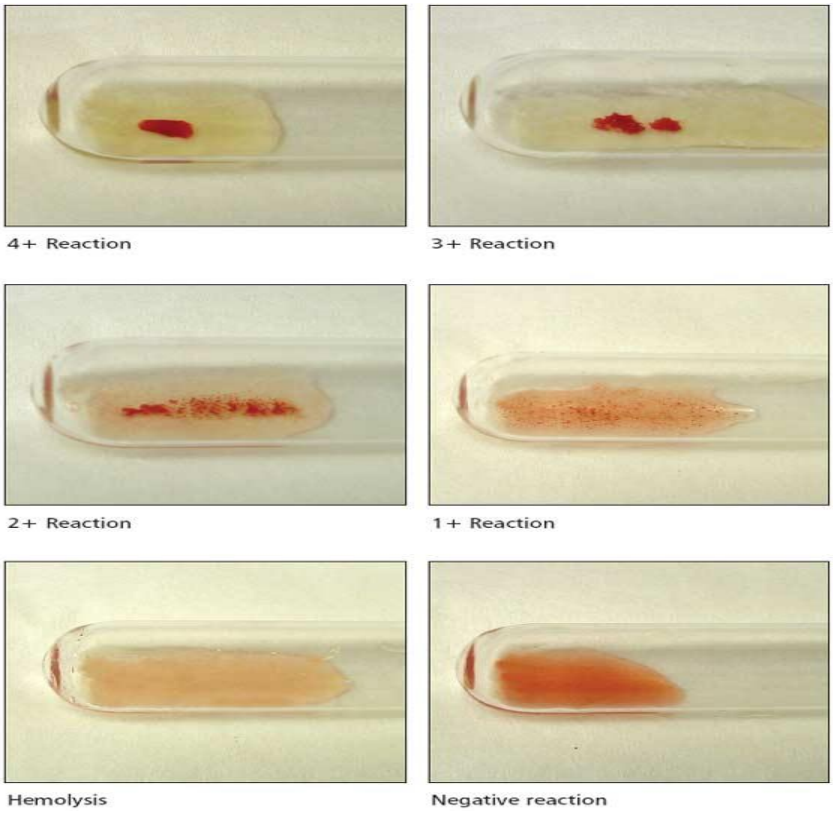


Figure.1 Grading of agglutination by tube method

Grades	Description
4+	1 big clump
3+	2 or 3 clumps
2+	many small clumps with clear supernatant
1+	many small clumps with turbid supernatant
w	granular suspension
Zero or -	smooth suspension
H	partial or complete hemolysis (positive reaction)

IV Rh D Phenotyping

Reagents

- Monoclonal Anti- D (IgM + IgG) Blend (D1 & D2 from two manufacturer)
- Monoclonal antisera (Anti-E, Anti-e, Anti-C, Anti-c)
- AHG (Anti-Human Globulin)
- 3% cell suspension of sample

PROCEDURE

Bench Preparation (NIB/BRL/SOP/44/R1)

- Prepare bench for performing the grouping
- Allow all reagents to come to Room Temperature.
- Fill in Performa for required details

Setting up tubes

Set 7 tubes for each sample as follows;

- 2 tubes labelled - D1 & D2
- 4 tubes labelled - C, c, E, e. for Phenotyping
- 1 control tube.

Rh D – Typing & Phenotyping

- Take 100ul each of Anti-D (D_1 & D_2) in respective tubes and add 100ul of 3% cell suspension to each tube
- Take 50ul each of Anti – C, c, E, e in respective tubes and add 50ul of 3% cell suspension to each tube.
- Mix well and centrifuge at 1000rpm for 1 min.
- Read agglutination & note the results in NIB/BRL/PRO/03/R1.
- Confirm all negative results under microscope & proceed for IAT.

Indirect Agglutination test (IAT) -Detection of weak – D antigen

- Incubate the negative test tube at 37^0 C for 30 minute.
- Centrifuge at 1000 rpm for 1 min. and observe the results
  - ..If positive, record the sample as D-Positive
  - .. If negative, wash the cells three times with normal saline, then decant completely.
  - Add 200 µl of AHG.
  - Mix the content of the tube and centrifuge at 1000 rpm for 1 minute.
  - Dislodge cell button by gentle shaking and observe the agglutination
  - If negative- record as Rh-D Negative; if positive – the sample is weak D variant.

INTERPRETATION OF RESULTS

	Antigen					Phenotype	Result
	D	C	c	E	e		
Rh(D) +ve Cell	+	+	0	0	+	DCe/DCe	R_1R_1
	+	0	+	+	0	DcE/DcE	R_2R_2
	+	0	+	0	+	Dce/dce	R_0r
	+	+	0	+	0	DCE/DCE	R_zR_z
	+	+	+	0	+	DCe/dce	R_1r
	+	0	+	+	+	DcE/dce	R_2r
	+	+	0	+	+	DCe/DCE	R_1R_z
	+	+	+	+	0	DcE/DCE	R_2R_z
	+	+	+	+	+	DCe/DcE	R_1R_2
Rh (D) Neg. Cell	0	+	0	0	+	dCe/dCe	r'r'
	0	0	+	+	0	dcE/dcE	r'' r''
	0	0	+	0	+	dcc/dce	rr
	0	+	0	+	0	dCE/dCE	r_yr_y
	0	+	+	0	+	dCe/dce	r'r
	0	0	+	+	+	dcE/dce	r''r
	0	+	0	+	+	dCe/dCE	$r'r_y$
	0	+	+	+	0	dcE/dCE	$r''r_y$
	0	+	+	+	+	dCe/dcE	r' r''

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LIST OF STANDARD OPERATING PROCEDURES

	OPERATING PROCEDURES (SOPs)	**BLOOD REAGENT LABORATORY**	COPIES	EFFECTIVE DATE	ISSUE DATE	PAGE NO (s)	REVISION STATUS
1	NIB/BRL/SOP/01/R1	Blood Sample Collection and Processing	2	24.2.2012	29.2.2012	8	1
2	NIB/BRL/SOP/02/R1	Grouping and Sub grouping of Blood Samples	2	09.2.2012	13.2.2012	9	1
3	NIB/BRL/SOP/03/R1	Rh- typing of Blood Samples	2	09.2.2012	13.2.2012	9	1
4	NIB/BRL/SOP/05/R1	Sample Receipt, Handling and Storage	2	24.2.2012	29.2.2012	5	1
5	NIB/BRL/SOP/34/R1	Operation & Maintenance of Bench Top Centrifuge	2	24.2.2012	16.3.2012	6	1
6	NIB/BRL/SOP/35/R1	Maintenance of Cold room (4oC)	2	29.2.2012	09.3.2012	5	1
7	NIB/BRL/SOP/36/R1	Preparation of Reagents & buffer	2	24.2.2012	09.3.2012	11	1
8	NIB/BRL/SOP/42/R1	Washing and Preparation of glassware	2	24.2.2012	29.2.2012	6	1
9	NIB/BRL/SOP/44/R1	Preparing and winding up of work bench	2	09.2.2012	13.2.2012	5	1

Proforma for Grouping and Sub – Grouping

Date of sample Collection: Sheet No.

Date of Testing:

Method	S. No	Lab. S. No	FORWARD GROUPING								REVERSE GROUPING				Blood Group
Forward Grouping: 1-Take 100ul of Anti-sera +100ul of 3% reagent red cells 2- Mix and centrifuge at1000 rpm for 1 minute 3- Note the agglutination			Anti							IAT	Reagent cells			Interpretation	
			A	B	AB	D_1	D_2	H	A_1		A	B	O		
Reverse Grouping: 1-Take 100 ul of serum + 50 ul of 3% known cells 2- Mix, centrifuge at1000 rpm for 1 minute 3 – Note the agglutination															
Criteria: i) 4+, One solid Agglutinate, ii)3+ , Several large Agglutination-Clear Background, iii) 2+ , Medium sized Agglutinates-Clear Background, iv) 1+ , small Agglutinates , v) W+ , Tiny Agglutinates Turbid Background vi) Negative- No Agglutination OR Haemolysis.															
Haemolysis considered positive in case of serum grouping when the test serum is used fresh Positive-Agglutination Negative-No agglutination															

Signature of Analyst: **Signature of Supervisor:**

Known Reagent Red Blood Cells used for Reverse Grouping:

Reagent RBC used (3%)	Lab. Reg S .No.	DOC

Reagents Used for Forward Grouping

S.No.	Reagent used	Vial No.	Manufacturer	Batch No.	Expiry date

Signature of Analyst: **Signature of Supervisor**

Proforma for Phenotyping

Date of sample Collection: **Date of Testing:** **Sheet No.**

Method	S. No.	Lab S. No.	Anti – D (Blend) D1	D2	Phenotype Group / Anti-Sera C	c	E	e	Antigen	Phenotype
1. Rh-D Typing Take 100ul each of Anti-D (D_1 & D_2) in respective tubes and add										
100ul of 3% cell suspension										
2. Rh-Phenotyping Take 50ul each of Anti – C, c, E, e in respective tubes and add										
50ul of 3% cell suspension to each tube.										
3. Centrifuge at 1000 rpm for one minute. Read reaction										
4. If negative for Rh-D proceed for IAT. Read reaction										
Interpretation agglutination:										
i) 4+, One solid Agglutinate,										
ii)3+ , Several large Agglutination-Clear Background, iii) 2+ , Medium sized Agglutinates-Clear Background, iv) 1+ , small Agglutinates , v) W+ , Tiny Agglutinates Turbid Background vi) Negative- No Agglutination OR Haemolysis.										

DETAILS OF REAGENTS USED

S.No.	Reagent used	Vial No.	Manufacturer	Batch No	Expiry date

Signature of Analyst **Signature of Supervisor**