In The Name Of God

The Chalenges of Interpreting Blood Group and Crossmatch Tests

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Headlines

- Introduction
- Standard ABO grouping
- ABO discrepancy
- Incompatible crossmatch
- Case presentation

Introduction

- **Blood transfusion is one of the most common processes during hospitalization.**
- **Determining blood type and cross match are critical pre-transfusion tests**
- **Tube method is the standard method for determining blood group.**
- **Any discrepant ABO group results should be resolved before blood is issued.**

A standard ABO grouping(tube test)

- **Evaluating requests for red cells**
- **Checking a patient history**
- **Evaluating patient sample**
- **Sample storage**
- **Interpretation of agglutination reactions**
- **Quality control**
- **Recognizing an ABO discrepancy**

REQUESTS FOR TRANSFUSION

- in oral, electronic, or written format.
- Two independent patient identifiers include the patient's first and last names and an ID number that is unique to the patient.

Don,t accept Requests for blood or blood components that:

l) lack the required information.

2) are inaccurate.

3) are illegible.

patient's history

- Diagnosis
  - -auto immune disease -Immunodeficiency disorders
  - -leukemia -Lymphoma
- a history of transfusion/transfusion reaction
- pregnancy
- Transplantation

Specimen Labeling

- label in the patient's presence.
- two independent patient identifiers.
- the date of the collection.
- the information on the label,on the wristband and the transfusion request should be equal.
- phlebotomist must be traceable.

SPECIMEN REQUIREMENTS

- either serum or plasma.
- plasma is often preferred.
- Hemolysis and Lipemic specimens create difficulties in evaluating test results.

- If a hemolyzed specimen is used, it should be noted in the patient's testing records to differentiate from hemolysis as a result of an antigen-antibody reaction.

Specimen Age

- **sample used for testing must be no more than 3 days old(Pregnancy, transfused within the prevaious 3 months)**
- **The day of collection is counted as Day 0.**
- **The recipient's and the donor's red cells must be stored at refrigerator temperature for at least 7 days.**

Reading and Interpreting Serologic Test Reactions

- hemolysis and/or agglutination shows antigen-antibody interaction.
- All personnel in a laboratory should use the same interpretations (eg, from 0 to 4+)
- A microscope can be useful for distinguishing rouleaux from true agglutination in tube methods.


Machine
Material
Person
QUALITY CONTROL
Method
Chain

Quality control

Reagent requirements

- should be clearly labeled with batch number, expiry date and storage temp.
- instructions for use should be enclosed with each reagent packing.
- All reagents & kit should be used according to the manufacturer's instructions.
- Use of positive & negative controls with each batch.
- All reagents must be carefully stored at recommended temp.

- NOTE: The date the vial is opened should be written on the vial

QC IN BLOOD GROUP SEROLOGY

- **reagents must be checked carefully to rule out any turbidity or contamination.**
- **Tubes should be labelled properly.**
- **Results should be recorded immediately after observation.**
- **Concave mirror (agglutination viewer) or microscope may be used to examine reactions that appear negative by the naked eye.**

Frequency of testing requirements

Reagent QC	Positive / Negative Control
ABO anti sera	Each day of use
Rh(D) anti sera	Each day of use
Other anti sera	Each day of use
AHG (IgG)	Each day of use
ABO reagent cells	Each day of use
ABS (antibody screening cells)	Each day of use

False negative or false positive results can occur from:

- • Bacterial or chemical contamination
- • Inadequate incubation time or temperature
- • Improper centrifugation
- • Improper storage of materials
- • Potency of the anti-sera or red cell reagent
- • Omission of tests reagents.

Corrective action

- 1.Review procedures
- 2.Search for recent events that could cause change

  -New reagent kit or lot number -New control bottle -Instrument maintenance -Instrument moved
- 3.Examine the environment conditions
- 4.Prepare new control material
- 5.Follow manufacturers trouble shooting guide
- 6.Contact manufacturers

DOCUMENTATION

If you have not documented it,

You have *NOT* done it.


A
B
O

ABO Group

- The 36 human blood group system.
- The ABO system initially described by Karl Landsteiner in 1900.
- the most important blood group system in transfusion and organ transplantation medicine.
- ABO antigens are found on red cells, platelets, and on tissues.

TABLE 12-1. Routine ABO Grouping

Reaction of Red Cells with Antisera (Red Cell Grouping)		Reaction of Serum with Reagent Red Cells (Serum Grouping)			Interpretation	Prevalence (%) in US Population	
Anti-A	Anti-B	A_1 Cells	B Cells	O Cells	ABO Group	European Ethnicity	African Ethnicity
0	0	+	+	0	O	45	49
+	0	0	+	0	A	40	27
0	+	+	0	0	B	11	20
+	+	0	0	0	AB	4	4
0	0	+	+	+	Bombay*	Rare	Rare

*H null phenotype (see section on H antigen).

+ = agglutination; 0 = no agglutination.

ABO Discrepancies

Possible Causes of ABO Typing Discrepancies

Category	Causes
Weak/missing red cell reactivity	ABO subgroup
	Leukemia/malignancy
	Transfusion
	Intrauterine fetal transfusion
	Transplantation
	Excessive soluble blood group substance
Extra red cell reactivity	Autoagglutinins/excess protein coating red cells
	Unwashed red cells: plasma proteins
	Unwashed red cells: antibody in patient's serum to reagent constituent
	Transplantation
	Acquired B antigen
	B(A) phenomenon
	Out-of-group transfusion

Possible Causes of ABO Typing Discrepancies

Mixed-field red cell reactivity	Recent transfusion
	Transplantation
	Fetomaternal hemorrhage
	Twin or dispermic (tetragametic) chimerism
Weak/missing serum reactivity	Age related (<4-6 months old, elderly)
	ABO subgroup
	Hypogammaglobulinemia
	Transplantation
Extra serum reactivity	Cold autoantibody
	Cold alloantibody
	Serum antibody to reagent constituent
	Excess serum protein
	Transfusion of plasma components
	Transplantation
	Infusion of intravenous immune globulin

Categories of ABO discrepancy

Type	Reasons	Conditions
Group I discrepancy	Weak reacting or missing antibodies.	-Chimerism (due to blood transfusion, transplanted bone marrow, exchange transfusion, feto maternal bleeding) -New born infants. -Elderly patients -Hypogammaglobulinemia (leukemia, immunodeficiency diseases)
Group II discrepancy	Weak reacting or missing antigens.	-Subgroups of A or B -Leukemia – excess amount of B, -Acquired B phenomenon (in gram negative septicemia, intestinal obstruction and cancer of colon or rectum).
Group III discrepancy	Protein/ plasma abnormality leading to rouleaux formation	-Elevated globulin level (in multiple myeloma, Waldenstrom' macroglobulinemia, plasma cell dyscrasias, Hodgkin lymphoma). -Plasma expanders like dextran, polyvinyl pyrrolidone –Wharton's jelly (in cord blood)
Group IV discrepancy	Miscellaneous problems	-Exposure of hidden erythrocyte T antigen (Polyagglutination) -Cold and warm autoantibody (AIHA) -Transfused foreign antigen. -Unexpected ABO iso-agglutinin and alloantibody. -Antibody other than anti-A & anti-B (E.g.: acriflavin antibody) -cis – AB individuals.

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Original Research Article

Resolving blood group discrepancy in patients of tertiary care centre in Odisha, India

Debasish Mishra[1]*, Pankaj Parida[2], Smita Mahapatra[2], Binay Bhusan Sahoo[3]

- **A total of 25,559 blood group**
- **57 blood group discrepancies (overall frequency 0.22%).**
- **20 (35.09%) cases of technical error**
- **37 (64.91%) cases of sample related error**

13.51%weak/missing antibody, 2.7% weak antigen expression, 2.7% rouleaux, 54.06% cold autoantibodies, 8.11%cold alloantibodies, 18.92%Bombay phenotype

Resolving ABO Discrepancies

- repeat the test with the same sample.
- testing washed red cells.
- testing a new sample.
- testing for unexpected red cell alloantibodies.
- reviewing the patient's medical record for conditions, medications, or recent transfusions.
- enhance antigen-antibody binding

  -incubating red cells at 4 C
- conducting adsorption and elution studies

Resolving ABO Discrepancies

- cold autoantibodies (cold autoadsorption,washing red cells with warm saline, dithiothreitol).
- Rouleaux (Saline replacement).
- cold-reacting alloantibodies (eg, anti-M).
- weak A subgroups with an antiAl.

- Patients with suspected B(A), acquired B, or A(B) phenotypes should be retested using different reagents.

ABO discrepancy

- **If you couldn,t solve the ABO discrepancy, which blood group would you suggest for transfusion?**

Group O with the same Rh of patient

TABLE 13-2. Prevalence of the Principal Rh Haplotypes

Fisher-Race Haplotype	Modified Wiener Haplotype	Prevalence (%)		
		White	Black	Asian
Rh positive				
DCe	R_1	42	17	70
DcE	R_2	14	11	21
Dce	R_0	4	44	3
DCE	R_Z	<0.01	<0.01	1
Rh negative				
ce	r	37	26	3
Ce	r′	2	2	2
cE	r″	1	<0.01	<0.01
CE	r^y	<0.01	<0.01	<0.01

Negative ab screening and incompatible IS crossmatch

- ABO incompatible
- Donor red cells are polyagglutinable
- Anti-A1 in serum of A2 & A2B
- Allo antibody (eg : anti-M)
- Roleaux formation
- Auto antibody (eg :anti-I)
- Passively acquired anti-A & anti-B

Negative ab screening and incompatible AHG crossmatch

- Donor red cell are DAT positive
- Dosage effect (anti-D) or variation in antigen strength (p1)
- An antibody to low incidence antibody
- Passively acquired anti-A & anti-B

Positive antibody screening & compatible crossmatch

- Anti-IH & non group O selected
- Ab demonstrating dosage and donor cell is heterozygote
- Lack of corresponding antigen

- **Positive ab screening & compatible crossmatch & negative auto control**

  Allo-antibody

- **Positive ab screening &in compatible crossmatch & positive auto control& negative DAT**

  - Ab to an ingredient in the enhancement media

  - Roleaux formation

Positive antibody screening & incompatible crossmatch & positive auto control& positive DAT

- Allo antibody causes DHTR
- Passively acquired autoantibody (IVIG)
- Cold or warm reactive auto antibody

SELECTING BLOOD FOR TRANSFUSION

- After an antibody detection, determine its clinical significance.
- Abs reactive at 37 C, in an IAT are potentially clinically significant.

there are many exceptions:

- anti-Vel, -P, and -PPlP k (-Tja) reactive only at cold temperatures yet may cause red cell destruction in vivo.

Anti-Ch, anti-Rg, and many of the Knops and Cost antibodies have little or no clinical significance

Selected Serologic Procedures

- **a pattern of weak reactions :the use of enhancement techniques or testing of panel cells treated with enzymes or chemicals. Destroy M, N, S,s, *Fya, Fyb,Xga, JMH, Ch, and Rg but* enhanced reactivity with other antibodies (eg, Rh, P, I,Kidd, and Lewis).**

- **LISS and PEG enhance autoantibodies**
- ***Temperature Reduction***

  Some antibodies (eg, anti-M, -N, -Pl, -Leb, and -Al)
- ***Increased Serum-to-Cell Ratio(4 to1)***
- ***Increased Incubation Time(not for LISS OR PEG)***

Case presentation

Case presentation

- A 34-year-old pregnant woman with O,Rh+ history.
- Candidate for IUT(intrauterine transfusion)
- Incompatible cross match with O,Rh-

Tube Testing

What,s your next step?

Anti-A	Anti-B	Anti-D1	Anti-D2	Rh control	A_1 Cell	B Cell
0	**0**	**4+**	**4+**	**0**	**4+**	**4+**

Antibody Screening

	Rh					Kell		Duffy		Kidd		Lewis		MNS				Luth		37 AHG
	D	C	c	E	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	M	N	S	s	Lu^a	Lu^b	
I	+	+	0	0	+	0	+	+	+	0	+	+	0	+	0	+	0	0	+	**0**
II	+	0	+	+	0	0	+	+	0	+	0	0	+	0	+	0	+	0	+	**2+**
III	0	0	+	0	+	+	+	0	+	+	0	0	+	0	+	+	+	0	+	**2+**
AC																				**0**

	Rh					Kell		Duffy		Kidd		Lewis		MNS				Luth		LISS AHG
	D	C	c	E	e	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	M	N	S	s	Lu^a	Lu^b	
1	+	+	0	0	+	0	+	0	+	+	+	0	+	0	+	+	+	0	+	o
2	+	+	0	0	+	0	+	+	0	+	+	+	0	0	+	0	+	0	+	o
3	+	0	+	+	0	0	+	0	+	0	+	+	0	+	+	0	+	0	+	2+
4	+	0	+	+	0	0	+	0	0	+	0	+	0	+	0	0	+	0	+	2+
5	+	+	+	+	+	+	0	0	+	+	0	0	+	+	+	0	+	0	+	1+
6	0	+	+	0	+	0	+	+	+	+	+	0	+	+	0	+	+	0	+	2+
7	0	0	+	+	+	0	+	0	+	+	0	0	+	+	+	0	+	0	+	2+
8	0	0	+	0	+	+	+	+	+	0	+	+	0	0	+	+	0	0	+	2+
9	+	0	+	0	+	0	+	0	0	0	+	0	0	+	+	0	+	+	+	2+
10	0	+	+	+	+	0	+	+	+	+	+	+	+	0	0	+	+	0	+	2+
11	+	+	0	0	0	0	+	+	0	0	0	+	0	0	+	0	0	0	+	o
AC																				o

Case presentation(cont)

- **Antibody of patient was anti-c.**
- **The patient's RBC phenotype was as follows: D+C+c-E-e+K- (R1R1& kell neg)**
- **O ,Rh positive and negative for E,c,Kell issued for IUT.**


You are never wrong to do the right thing!


Thank you