Immuno-haematological testing in neonate and paediatric age group

Neonates are not small adults!!

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Introduction

- Physiologically neonates / infants are not small adults
- Their immune system is immature
- Red cell antibody production starts at 4 months of age
- Humoral immunity is maternally derived
- Maternal IgG antibody crosses placenta and remain in circulation till 3 – 4 months
- Expression of red cell antigens on RBCs is weak

Implications for Transfusion Medicine

- Problems in forward and reverse grouping
- Cross matching issues (compatibility with mother)

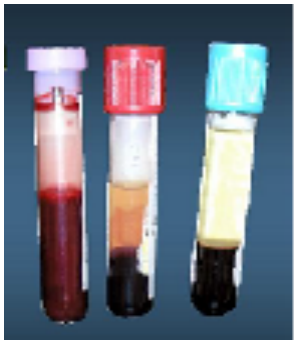
A case study

- Blood centre received request for exchange transfusion with 1 ml of blood in EDTA
- Age / Gender: 2 days old male infant
- Clinical details
  - Hyperbilirubinemia
  - Anaemia
  - Hypoproteinemia
- Maternal blood sample was request
  - Maternal sample in EDTA

Why maternal sample needed

- ABO antigens poorly expressed and corresponding ABO antibodies not developed till 4 months of age.
- Blood group confirmation by reverse group is unreliable
- Maternal IgG ABO antibodies are detected in neonate.
- Infant may have paternally inherited antigens foreign to mom
- Protocol for IH testing
  - ABO / Rh — maternal and infant sample
  - Antibody screen — maternal serum
  - DAT — Infant red cells
  - Cross match — compatible with maternal serum

Volume of blood sample



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even 7 ml loss of blood = loss of 10% of blood volume

Minimum volume of sample required

Age of patient	Minimum volume of sample
Neonates (up to 4 months)	0.5 ml EDTA + 6 ml maternal sample
4 months to 3 years	3 ml EDTA
> 3 years	6 ml EDTA

Australian Transfusion Services Guidelines

Continuing with our case....

Lab investigations

- Hb 9.5 gm/dl
- Bilirubin: 20 mg/dl

History

- The mother is G2P2
- Previous pregnancy- 2 years ago
- First born child was also affected
- No history of transfusion

Continuing with case - ABO/Rh

Mother

Forward Grouping			Reverse Grouping	
Anti-A	Anti-B	Anti-D	A_1 Cells	B Cells
0	0	4 +	+4	+4

Group O pos

Baby

Forward Grouping			Reverse Grouping	
Anti-A	Anti-B	Anti-D	A_1 Cells	B Cells
+ 3	0	3 +	0	0

Group Forward A Reverse AB

Continuing with case....

Why the discrepancy in baby's blood grouping?

- Type II Discrepancy – missing antibody
- Baby's serum doesn't have ABO antibodies to react with the reagent red cells on reverse grouping
- Maternally derived ABO antibodies are IgG type which will not react in saline phase
- No reaction interpreted as AB group on reverse
- While forward is A group

Implication for IH Testing:
So as a rule only forward grouping is done for neonates and infants up to 4 months of age

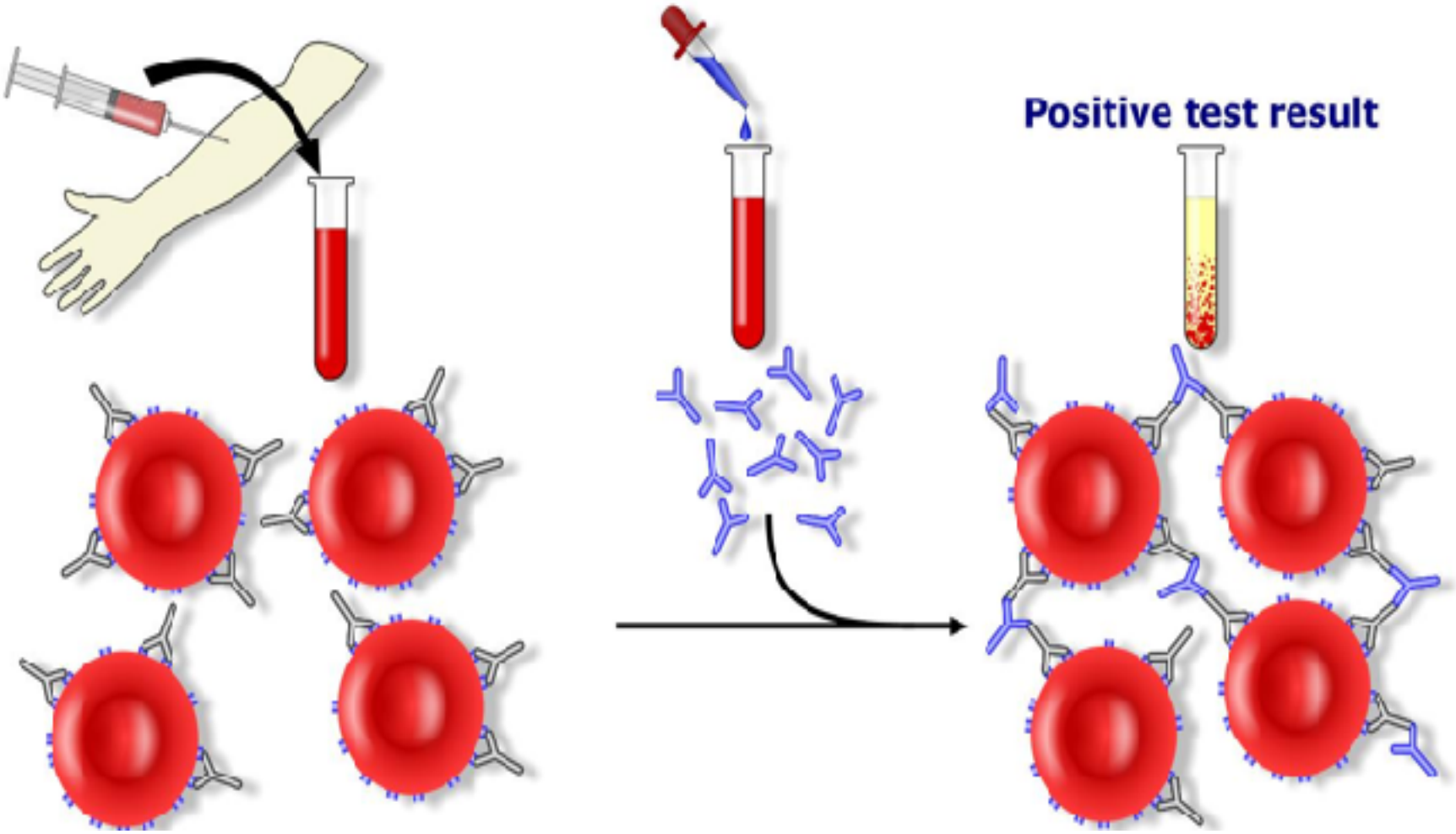
Continuing with case.... Story so far

- 2 days old infant with hyperbilirubinemia needed exchange transfusion.
- Mother is group O pos while baby A pos
- Laboratory markers of hemolysis present
  - Hb decreased
  - Retic & LDH increased
  - Bilirubin elevated
  - Smear spherocytes and normoblasts

What Next:

Determine nature of hemolysis - immune or non-immune

Direct Antiglobulin Test (DAT)



Common causes of Pos DAT in neonates

- ABO HDN (Commonest cause of pos DAT in new born)
- HDN due to Rh and non Rh antibodies
- Non-specifically adsorbed proteins such as
  - High-dose intravenous immune globulin
- Passively acquired alloantibodies from plasma transfusion
- Complement activation due to bacterial infection
- Sickle cell disease / β-thalassemia
- Drug induced Antibodies

Interpretation of DAT in neonates

DAT on neonate	Maternal Ab status	ABO in-compatibilty	Interpretation
Positive	Negative	Yes	+ DAT due to ABO Ab
Positive	Clinically significant Ab	No	+ DAT due to maternal alloantibody (RhD)
positive	Clinically significant Ab	Yes	+ DAT due to maternal allo-Ab and/or ABO Ab
Negative	Negative	Yes	HDN due to anti-A,B can not be ruled out

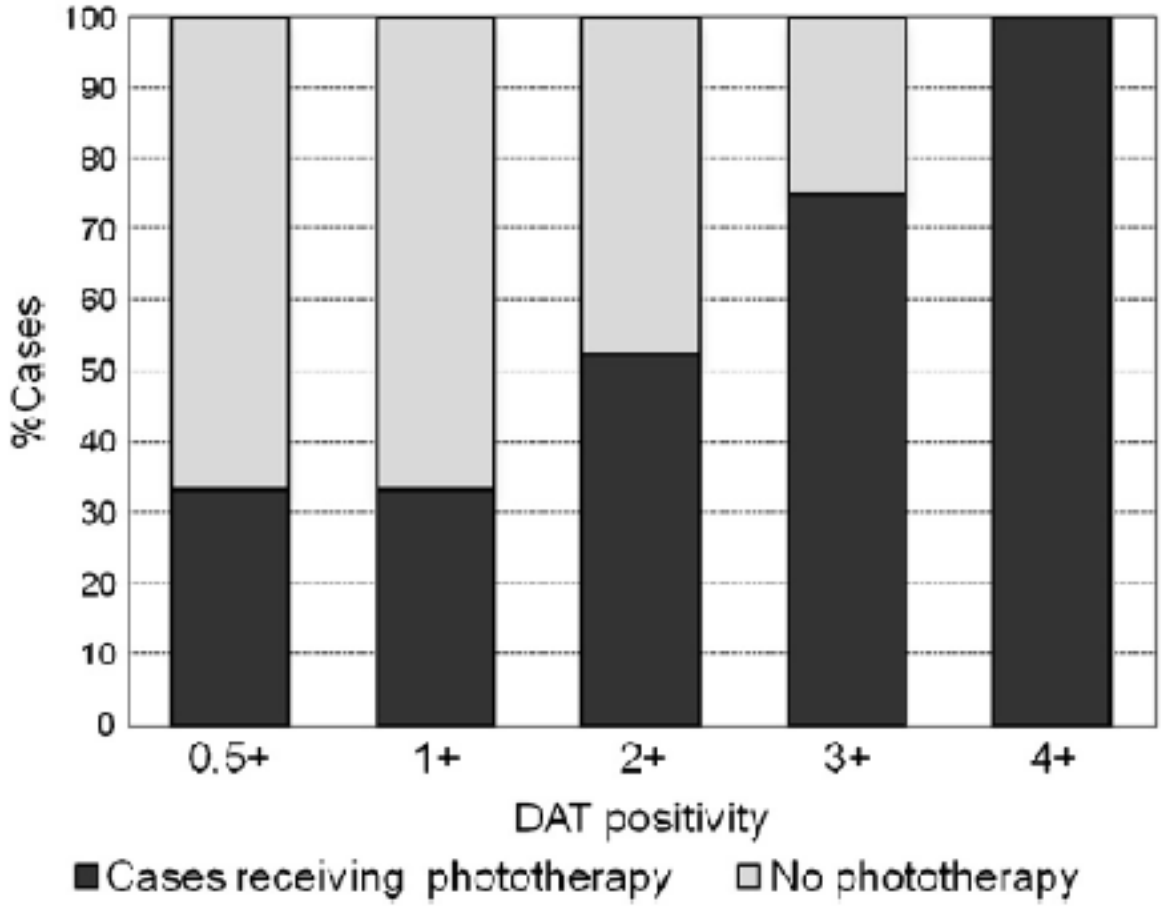
Importance of DAT in Immune Hyperbilirubinemia

Study	Infants screened	DAT pos	PPV %	NPV %
Meberg et al	2463	100	12	96
Herschel et al	660	23	53	89
Dinesh et al	1724	94	23	92

ORIGINAL ARTICLE

Importance of Direct Antiglobulin Test (DAT) in Cord Blood: Causes of DAT (+) in a Cohort Study

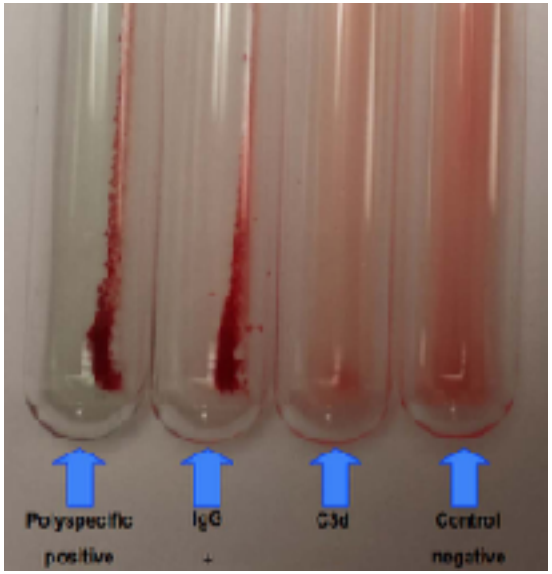
Ped & Neonatol 2015, 56: 256



Correlation of increasing strength of DAT positivity with phototherapy need

Continuing with case....

- DAT on infant red cells: 2 +
- Monospecific DAT: IgG
- IgG subtype: IgG2



Interpretation

Hemolysis is immune mediated

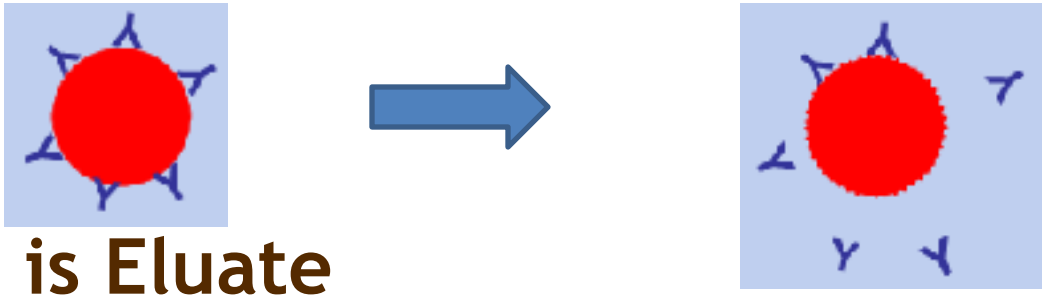
- ABO HDN
- Rh HDN
- HDN due to non Rh, non ABO antibody

What Next

- **Elution on infant red cells**
- **Antibody screen in mother serum**

What Next -Elution on infant DAT + cells

- **What is elution**
  - Process to remove antibodies (usually IgG) that are sensitizing RBC from RBC surface.



- **What is Eluate**
  - A fluid medium containing the antibodies that have been deliberately removed from RBCs, allowing for antibody identification.

Elution – applications

- Investigation of a positive DAT
  - Requires total elution, in which the RBCs are completely destroyed
  - Eluate is tested with panel cells for antibody identification
  - Useful in HDFN, HTR & AIHA
  -
- Preparation of antibody-free RBCs for use in phenotyping or autologous adsorption studies

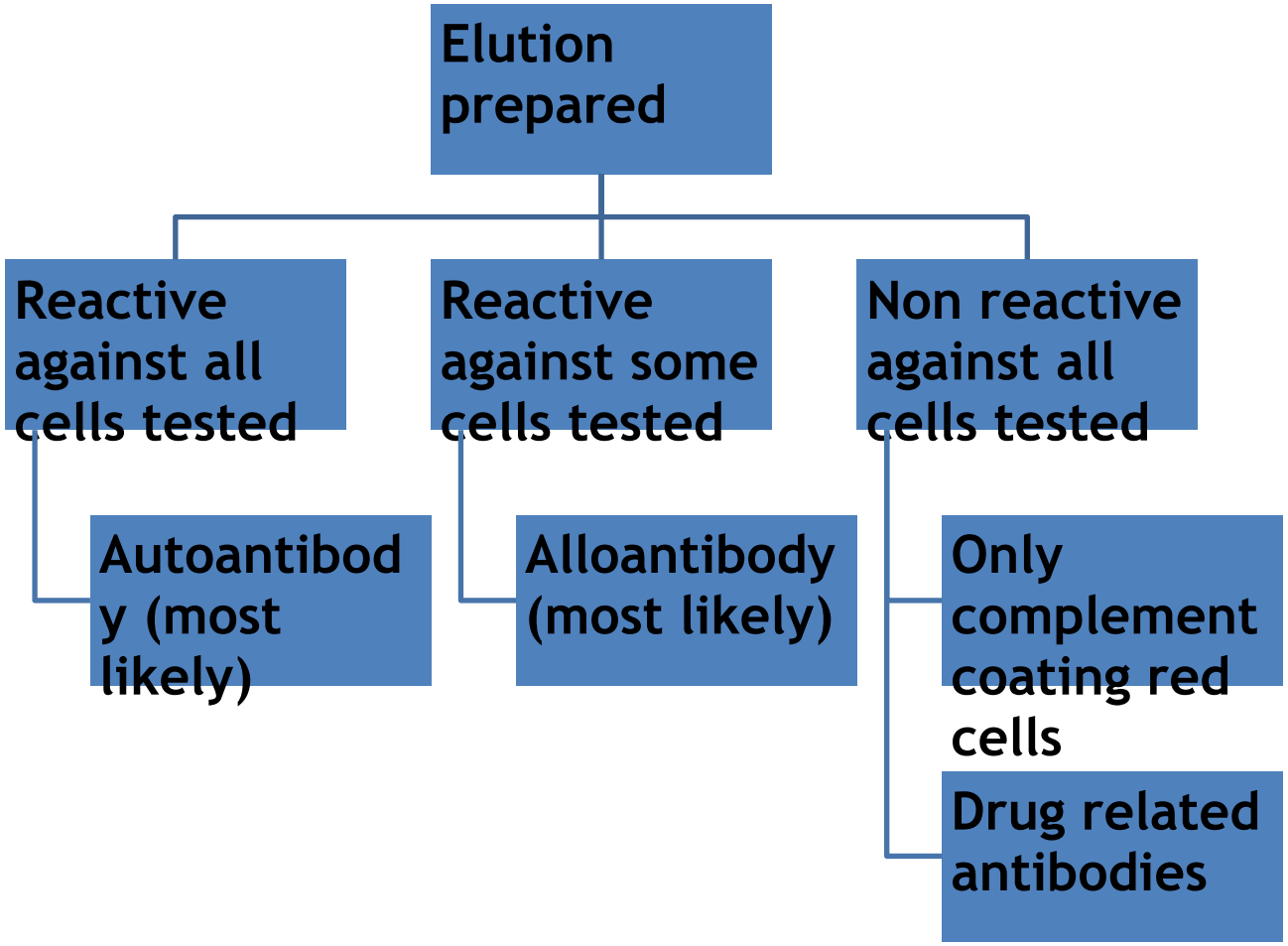
Mechanism of elution methods

- Alteration of thermodynamics using heat or cold temp
- Alteration of membrane structure using acids
- Reversal of attractive forces between antigen and antibody

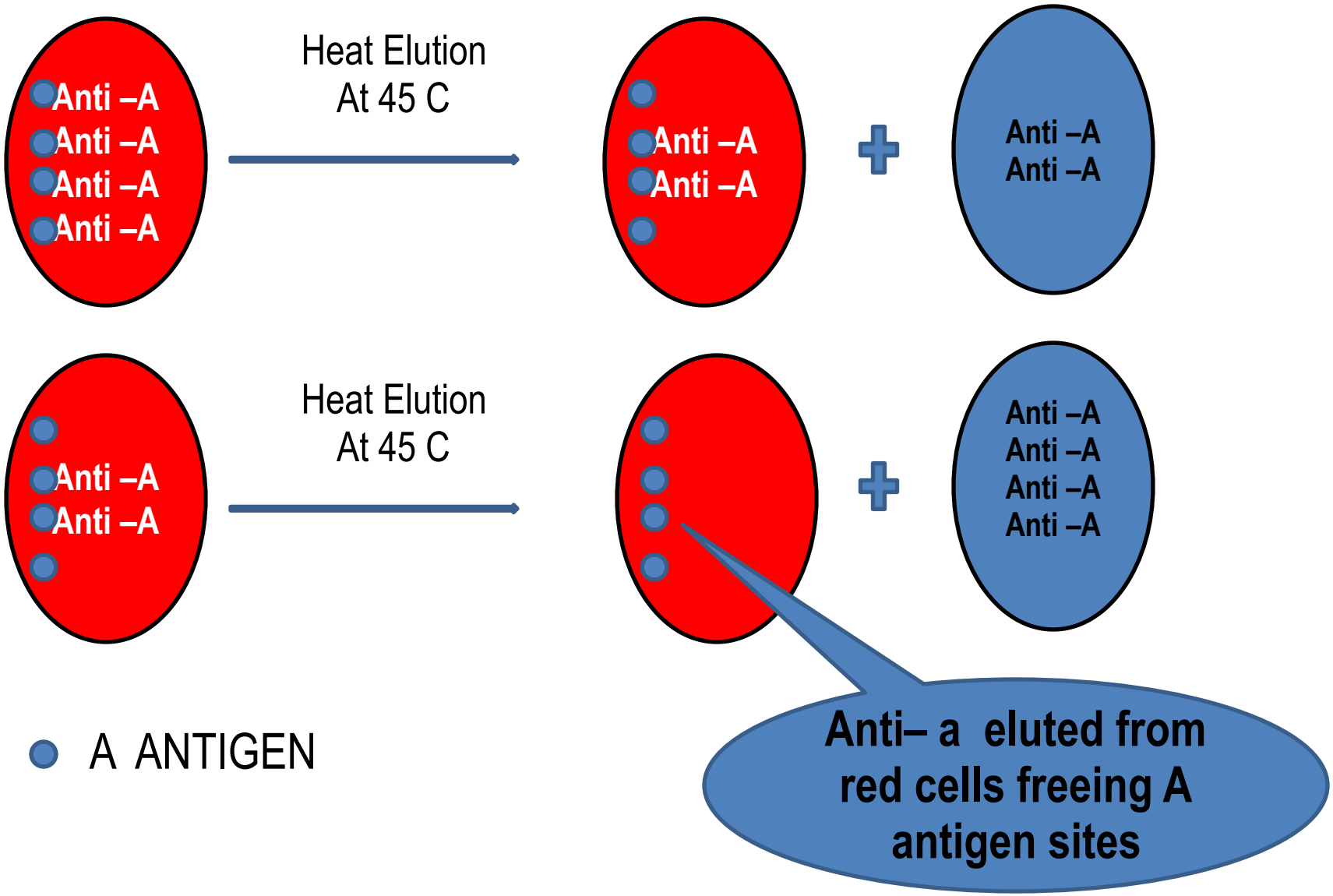
Common elution methods

Method	Use	Benefits	Pitfalls
Heat elution	ABO HDN	Easy	Not useful in IgG agglutinating Ab
Freeze thaw	ABO HDN	Quick Small volume required	Not useful for other Ab
Acid elution	Warm auto and alloAb	Easy	Possible false positive eluate when high titer Ab present
Cold acid elution	Warm auto and alloAb		Reagent preparation Acidity may cause red cell hemolysis

Elution result interpretation



Elution in the present case



Continuing with the case……

- **Story so far**
  - 2 days old infant with significant hyperbilirubinemia
  - Laboratory evidence of hemolysis
  - Immune hemolysis – DAT +
  - Fetomaternal ABO incompatibility present
  - Eluate on infant red cells demonstrate anti-A IgG
- **What next**
  - Antibody screen on maternal serum & titer
  - Compatibility testing
  - Prepare unit for exchange transfusion

Antibody Screening in mom's serum

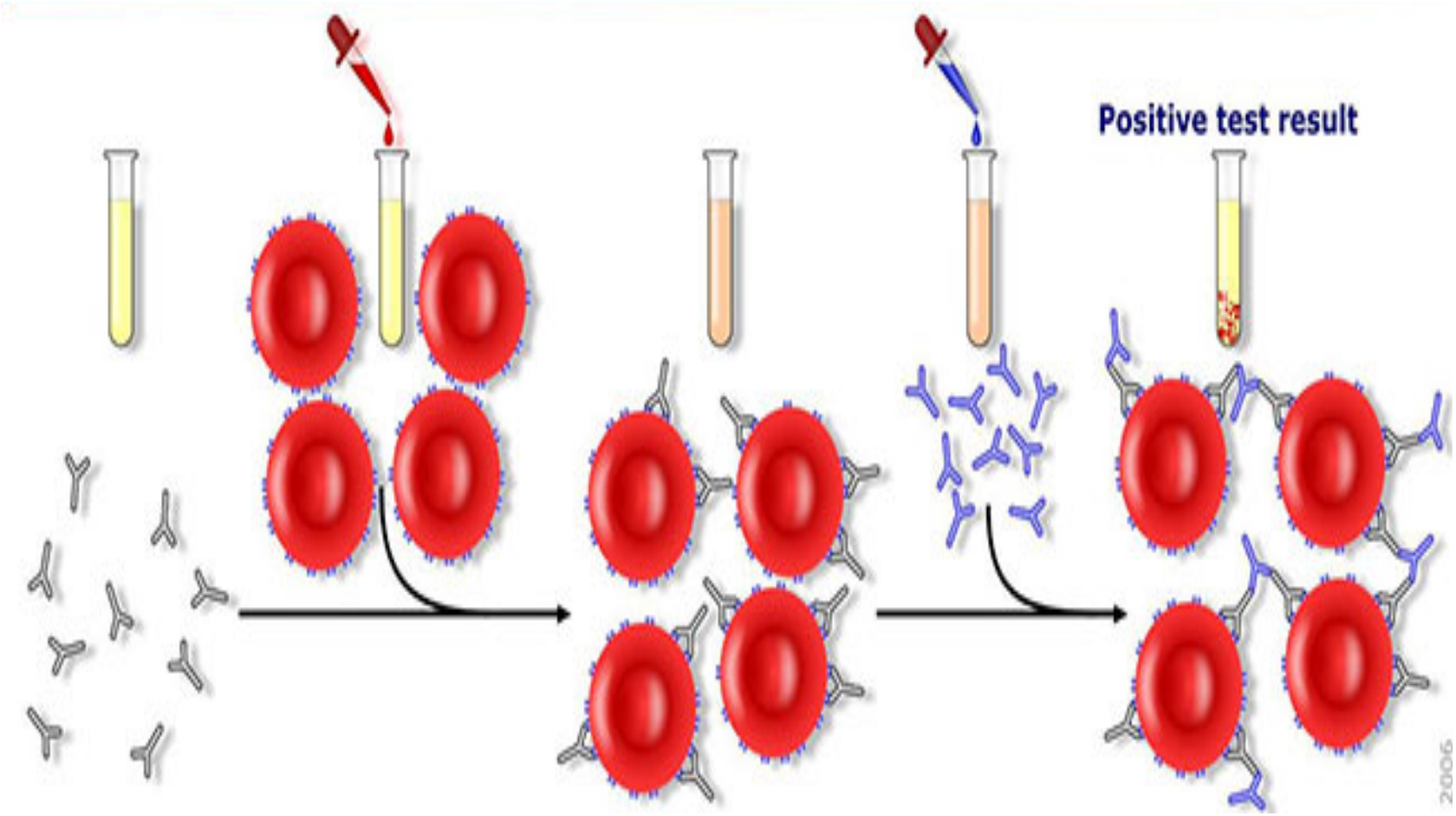
- Purpose is to detect red blood cell antibodies other than anti-A or anti-B.
- "Unexpected" because only 0.3 to 2 % of the general population have positive antibody screen.
- Once an unexpected antibody is detected, antibody identification studies are performed to determine the antibodies specificity and clinical significance.

Antibody Screening on mom's serum

- Antibody screening involve testing mother serum against reagent red blood screening cells
- Screening cells are commercially prepared group O cells obtained from individual donors that are phenotype for the clinically important red blood cell antigens.
- Group O cells are used so that naturally occurring anti-A or anti-B will not interfere with detection of unexpected antibodies.
- The cells are selected so that the following antigens are present on at least one of the cell sample;

  D, C, E, c, e, M N, S, s, P, Lea, Leb, K, k, Fya, Fyb, and Jkb.

Indirect Antiglobulin Test



Interpretation of antibody tests

Cell	Rh-hr								MNS				Kell				P	Lewis		Duffy		Kidd		Others	Cell	Results
	D	C	E	c	e	f	C^w	V	M	N	S	s	K	k	Kp^a	Js^a	P1	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b			37°C AHG
1	+	+	0	0	+	0	0	0	+	0	0	+	0	+	0	0	+	0	+	+	+	0	+	Rg(a+)	1	
2	+	+	0	0	+	0	+	0	+	+	+	0	0	+	0	0	+	0	0	0	0	+	0		2	
3	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	0	0	+	0	0	+	+	+		3	
4	0	+	0	+	+	+	0	0	+	0	+	+	0	+	0	0	+	0	+	+	0	+	0		4	
5	0	0	+	+	+	+	0	0	0	+	+	+	0	+	0	0	+	0	+	0	+	0	+		5	
6	0	0	0	+	+	+	0	0	+	0	+	0	+	+	0	0	+	0	+	+	0	0	+		6	
7	0	0	0	I	I	I	0	0	I	I	I	I	0	I	0	0	I	0	I	0	I	I	0		7	
8	+	0	0	+	+	+	0	+	0	+	0	0	0	+	0	0	+	0	0	0	0	0	+		8	
9	0	0	0	+	+	+	0	0	+	+	+	+	+	0	0	0	0	+	0	+	0	+	+		9	
10	0	0	0	I	I	I	0	0	I	0	0	I	I	I	I	0	I	0	0	0	I	I	I	Yt(b-)	10	
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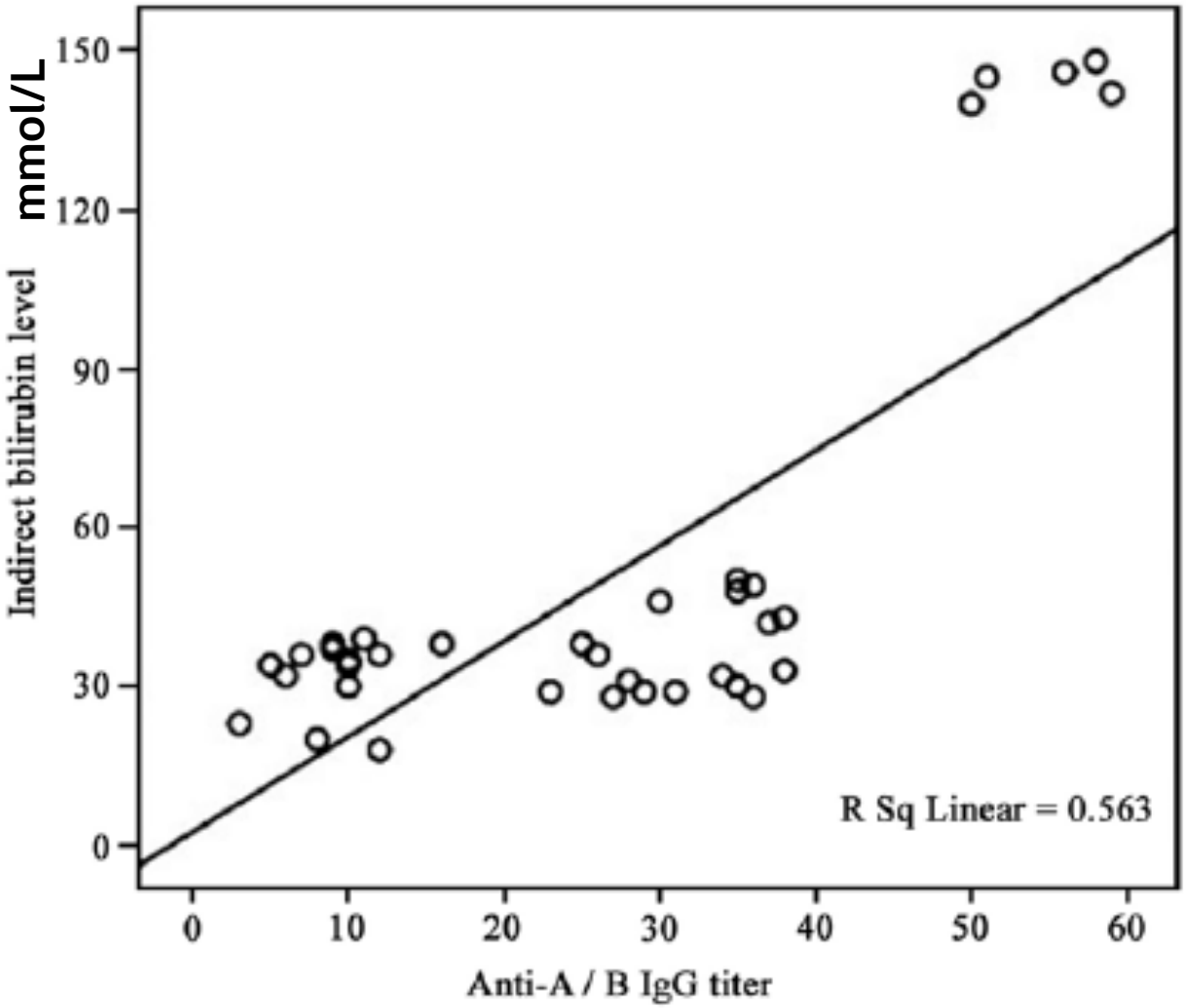
Original Article

Clinical study of the relationship between prenatal antibody titer and hemolytic disease of newborn



Int J Clin Exp Med 201

Diagnostic criteria for Immune HDN

- Blood group incompatibilities
  - Mother with known red cell alloimmunization (eg anti-D, anti-K)
  - Non O infants born to group O mother
- Laboratory evidence of hemolysis
- Demonstration of red cell coating with antibodies by a positive DAT

ABO Haemolytic Disease

- Limited to mothers who are blood group type O and whose babies are group A or B.
- More common than Rh HDN but is usually milder and rarely responsible for fetal deaths.
- Unlike Rh disease, ABO HDN may affect the firstborn ABO-incompatible infant since anti-A and anti-B antibodies are present normally in Group O adults.
- These naturally occurring antibodies are probably secondary to sensitization against A or B antigens in food or bacteria.

Prevalence of ABO HDN

- The low incidence may be due to the fact that most anti-A and anti-B antibodies are of the IgM type and do not cross the placenta.
- Only a a small proportion of Group O individuals produce anti-A , anti-B antibodies of the IgG type capable of crossing the placenta
- In addition, there are only a small number of fully developed A or B antigen sites on fetal and neonatal RBCs.
- The effect of anti-A and anti-B antibodies on red cells is also diluted by other tissues bearing these surface antigens.

Comparison of Rh vs ABO HDN

Blood group and antibodies		
	Rh HDN	ABO HDN
• Blood group	Rh	ABO
• Mother	Negative	O
• Infant	Positive	A or B
• Type of antibody	IgG1 / IgG3	IgG2
• Maternal antibodies	Always present	Not clear
• DAT on Infant red cell	++++	+

Comparison of Rh vs ABO HDN

Clinical Aspects		
	Rh HDN	ABO HDN
• Occurrence in first child	<5 %	40- 50 %
• Severity in next pregnancy	Severe	No
• Stillbirth & /or Hydrops	Frequent	Rare
• Severe anaemia	Frequent	Rare
• Degree of jaundice	+++	+
• Hepatosplenomegaly	+++	+

Compatibility testing in neonates

Lab Testing		Recommendation
Group & DAT on neonate	Group & antibody screen on mother	
Negative	Negative	Issue blood on demand after X match. No further compatibility testing till 4 months in same hospital
Positive	Positive / Negative	Full compatibility testing using maternal serum

If the baby is discharged and readmitted a new Group and DAT sample is required

Exchange Transfusion

- Assessing the need for exchange transfusion depend upon followings
  - Total bilirubin level
  - Haemoglobin level
  - Clinical symptoms

Exchange Transfusion

- Guidelines suggest exchange transfusion in the following circumstances:
- **Within 12 hours of birth if:**
  - Cord blood bil > 3 to 5 mg/dL for preterm infants
  - Cord blood bil > 5 to 7 mg/dL for term infants
  - Rate of increase is > 0.5 mg/dL/hour
  - Hb <10 g/dL combined with hyper-bilirubinemia
- **After 24 hours of birth if:**
  - Total bil > 20 mg/dL
  - Bilirubin increase of > 0.5 mg/dL/hour
  - Hb <10 g/dL combined with hyper-bilirubinemia

Rossi Pricnciple of TM 5th Ed

How it works?

- Exchange transfusions supply the neonate with compatible red cells and fresh plasma
- Incompatible red cells, bilirubin, and maternal antibodies in plasma are removed
- A standard exchange transfusion of twice the infant's blood volume
  - Reduces incompatible fetal red cells by about 85%
  - Bilirubin and maternal antibody concentrations are reduced by 25% to 45%

Preparing a unit for ET

- Group O or blood group compatible
- < 5 days old
- Hct 45 – 60% adjusted with AB Plasm
- SAGM removed
- Leukofiltered
- Irradiated
- Hb S negative
- Volume 170 ml/Kg in term and 200 ml/Kg in pre term

Closing our case

- Diagnosis : ABO hemolytic disease
- Feto maternal incompatibiliy
  - Mom group O, infant A
- Lab evidence of immune hemolysis
- Significant anemia and hyperbilirubinemia
- Eluate on infant red cells demonstrated anti-A IgG antibodies
- Compatible blood with maternal serum provided for exchange transfusion

Practice guidelines for prenatal and perinatal immunohematology, revisited

Transfusion 2001, vol 41

W. John Judd, for the Scientific Section Coordinating Committee of the AABB

Situation	Testing protocol
Infant born to D negative mothers	ABO, RhD, weak D
Infants born to mothers with clinically significant antibody	ABO, RhD, DAT
Infants born to mothers with no significant antibody AND Infant with signs & symptoms of HDN	ABO, RhD, DAT
	If ABO incompatibility exists, infant eluate tested for IgG anti-A, anti-B
	If no ABO incompatibility, maternal serum or infant eluate tested against paternal red cells

Concluding remarks

- Immunohematological testing in neonatal and pediatric patients is crucial for proper diagnosis and management
- Essential to have advanced IH labs in all blood centers
- Awareness amongst pediatricians regarding testing protocols, sample requirements is important