Serological Tools For Investigating Problems In Immunohaematology

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• Immunohaematology is an application of the principles of Immunology to the study of red cell antigens and their corresponding antibodies in blood for resolving the problems.
Purpose

Important overall purpose of the field of Immunohaematology is to predict a successful transfusion outcome
Outcome

Pretransfusion testings to ensure the best possible results in blood transfusion i.e. the transfused red blood cells will have an acceptable survival rate.
Detection of antigens or antibodies

- Agglutination
  - Sensitization
  - Visible agglutination

- Neutralization
  - Used in secretory studies
Basic Laboratory Investigations

- ABO and Rh Blood Grouping
- Crossmatching
- Detection of unexpected antibodies
- Selection of compatible units
• Early part of 20\textsuperscript{th} century, many important discoveries leading to safer compatibility procedures

• Latter part of the century saw developments in serological methodology.

• Molecular biology in Immunohaematology.
Importance of blood group systems

• Blood group antibodies play an important role in transfusion medicine - in blood transfusion and pregnancy. Though all antibodies are not clinically significant many have the potential to be clinically significant.

• Importance of the system is based on whether the antibodies can facilitate accelerated destruction of red cells having the corresponding antigen.

• Unlike ABO & Rh, other blood group antigens are not tested for in blood banks as their antibodies are not of frequent occurrence.
ABO GROUPING

- Cell and serum grouping is a must
- Cell grouping with standard antisera and serum grouping with pooled A, B and O cells
- Any discrepancy to be resolved
Problems due to discrepancies in cell & serum grouping

- Technical errors
  - clerical errors
  - dysfunction of reagents or equipments
  - procedural errors

- RBC grouping & serum grouping
Hemolysis

• Detected in serum after centrifugation (red)

• Can result from:
  -- Complement binding
    Anti-A, anti-B, anti-H, and anti-Lea
  -- Bacterial contamination
Problems due to discrepancies in cell & serum grouping

* Problems in RBC grouping
  a. Weak reacting or missing antigens
  b. Unexpected antigen reactions

* Problems in serum grouping
  a. Weak reacting or missing antibody
  b. Unexpected antibody reaction
Problems in RBC grouping

➢ *Weak reacting or missing antigens*

1. Subgroups of A & B
2. Alteration of antigen expression due to some disease conditions
3. Chimerism

Contd.......
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- Group O
- Group A

- A missing antigen in the forward grouping
Weaker variants of A and B

- Subgroups weaker than A₂ termed weaker variant or weak subgroups. They are A₃, Ax, Am, Ael etc.


- Subgroups of B are rarer than subgroups of A. They are B₃, Bx, Bm etc.

*Identifying weaker subgroups essential for donor population*
Bombay phenotype

- First reported by Bhende et al in Bombay in 1952
- Red cells lack A, B and H antigens while serum shows presence of anti-A, anti-B and anti-H
- Confirmation by absorption-elution with anti-A, anti-B, anti-AB and anti-H, secretor status and serum inhibition
- Test family members to detect any other member with this rare group
- Cryopreservation of donor red cells
Unexpected antigen reactions

1. Rouleaux due to elevated levels of globulin
2. Antibody coated red cells leading to positive DAT
3. Polyagglutination
4. Acquired B antigen
Resolving acquired B

- Check patient’s diagnosis: infection?
- Some anti-B reagents do not react with acquired B
- Test patient’s serum with their own RBCs
  - The patient’s own anti-B will not react with the acquired B antigen on their red cell (autologous testing)
- Test the red cells with anti-B reagent acidified to pH 6.0
Problems in serum grouping

Weak reacting or missing antibody

- New born infants
- Elderly people
- Hypo or agammaglobulinemia
- Prozone/high titre antibody
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- **Group A**
- **Group AB**

- Missing or weak antibodies
Unexpected antibody reactions

- Rouleaux
- \(A_2\) and \(A_2B\) with naturally occurring anti-\(A_1\)
- Cold autoantibodies like anti-I
- Cold alloantibodies like anti-M, anti-N etc
- Passively acquired antibodies
Rouleaux

- Can cause both extra antigens and extra antibodies
- “Stack of coins” appearance
- Falsely appear as agglutination due to the increase of serum proteins (globulins)
- Stronger at IS and weak reaction at 37°C and no agglutination at AHG phase
- Associated with:
  - Multiple myeloma
  - Waldenstrom’s macroglobulinemia
  - Hydroxyethyl starch (HES), dextran, etc.
Resolving Rouleaux

• Remove proteins

• If the forward grouping is affected, wash cells to remove protein and repeat test

• If the reverse grouping is affected, perform saline replacement technique
Determination of Rh D status

• Using anti-D typing reagents

  Monoclonal reagents are widely used now

• Available as IgM, Blend of IgM + IgG and IgG

• IgM reagents used for immediate spin testing

• Blend of IgM + IgG to perform antiglobulin test when the immediate spin test result is negative
Rh Blood Grouping

- Techniques as per the reagents used
- If red cells DAT positive, interpretation of results with caution
- Discrepancy in Rh grouping results while dealing with Rh D variants
Variants of Rh D antigen

- Quantitative variants - characterized by lower or higher number of D antigenic sites (weak D, D -- - )

- Qualitative variants - characterized by the absence of one or more epitopes (partial D)

- D antigen is made up of different parts or antigenic determinants called epitopes.

- 37 epitopes of D antigen known

Cont’d.........
• Weak D - All epitopes present but with reduced expression of D antigen

• Partial D - Some epitopes missing
  May produce antibody against the missing epitopes if exposed to them
## Typing with Anti-D Reagents and their Interpretations

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Reagents to be used to detect D variants (Partial D & Weak D) among donors, recipients of blood transfusion and antenatal women - Recommendations

Recipients & Pregnant Women

• Limited specificity anti-D reagent (IgM monoclonal D)
• Do not perform IAT

Blood donors & Cord Blood Samples

• Broad specificity anti- D reagent (IgM + IgG)
• Perform IAT
## Reaction profile of the ALBAclone Advanced Partial D typing Kit

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Diagnostic Strategy For Identification Of D Variants In Indian Population

Identification of D variants in routine RhD typing

Use of two anti-D reagents – cell line LHM 70/45, any one of the cell lines LHM 76/59, ESD-1, LHM 76/55

If D variant - Discrepant results

Further characterization of D variants using panel of anti-D reagents and molecular genotyping by specialized labs

Kulkarni S, Vasantha K et al. A simple diagnostic strategy for RhD typing in discrepant cases in Indian population Blood Transfus 2012
Diagnostic Strategy For Identification Of D Variants In Indian Population

RhD discrepancies

Test for “C” antigen

If positive for ‘C’ antigen test for D antigen with cell line LHM 70/45

Negative Partial D variant

Further characterization using panel of epitope specific monoclonal anti-D and molecular genotyping by specialized laboratories
Resolving incompatibilities in the major Crossmatch

Causes of positive results in the major cross match

- Incorrect ABO grouping of donor or patient
- Alloantibody in patient’s serum
  - Test with panel cells
    - If all donors incompatible, suspect antibody against high frequency antigen or multiple antibodies
    - If only one donor incompatible may be antibody against low frequency antigen
    - If antibody screen negative, serum may contain anti-A$_1$ or passively acquired agglutinins
• Could be due to the presence of autoantibodies
  - Against high incidence antigens – compatible unit is difficult to find
    - Any underlying alloantibody to be identified

• Donor unit may be DAT positive
  - Incompatibility with many recipients serum samples in the IAT phase – DO NOT USE THE UNIT
Transfusion of non group specific blood

• When units of an ABO group other than patient’s own type have been transfused, additional units should be selected after testing for the presence of anti-A and anti-B in recipient’s fresh sample.

• If serum compatible with patient’s own RBCs in the antiglobulin phase, then group specific blood may be given.

• If the AHG phase shows incompatibility, then transfusions to continue with alternate blood group.
Antibody Detection

- A key process in pretransfusion compatibility testing
- Detection and monitoring of patients at risk of delivering infants with HDN
- Investigation of potential hemolytic transfusion reactions and immune hemolytic anemias.
Antibody Screen

- Antibody screening tests involve testing patient’s serum against two or three reagent RBC samples called screening cells.
- Screening cells commercially available or can be prepared in house.
- Antibody screening cells are of group O.
- These cells phenotyped for the most commonly encountered and clinically important RBC antigens.
- Homozygous expression of the antigens for detection of antibodies that show dosage.
# Example of a 3 cell antibody screening cell set

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Direct Antiglobulin Test

- Direct anti-globulin test on patient’s red cells to differentiate allo and auto antibodies
  - DAT is negative in alloantibodies
  - DAT is positive in autoantibodies

- Auto control
Interpretation of the Antibody Screening results based on:

- Phase of reaction
- Autocontrol
- With how many screening cells the reaction was seen
Methods for Antibody Screening

- Manual
- Semiautomation
- Automation
Antibody Identification

• When antibodies are detected, they must be identified

• Antibody identification needed for transfusion purposes and it is an important part of compatibility testing

• A person with antibody when exposed to donor cells with the corresponding antigen serious side effects can occur
Antibody Panel

- An antibody panel usually includes at least 10 panel cells:

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Patient Typing

INTERPRETATION:
Techniques for Antibody Screening & Identification

- Saline
- Enzyme
- Albumin
- Indirect Antiglobulin Technique
  - NISS
  - LISS
  - PEG
- Column Agglutination Technology
- Solid Phase Technology
An antibody will only react with cells that have the corresponding antigen

Antibodies will not react with cells that do not have the antigen
Individuals do not make alloantibodies against antigens they have
• Antigen typing of patient’s red cells aids in the confirmation of the specificity of the antibody

• This can be done only if the patient has not been recently transfused
Antibody Titration

• Antibody titration

- To quantify amount of antibody
- Useful in obstetric patients having IgG antibody which may cause HDN
Neutralization

• Common substances
  – $P_1$ substance (sometimes derived from hydatid cyst fluid)
  – $Le^a$ and $Le^b$ substance (soluble antigen found in plasma and saliva)
  – $I$ substance can be found in breast milk
  – $Sd^a$ substance derived from human or guinea pig urine

**many of these substances neutralize COLD antibodies; Cold antibodies can sometimes mask more clinically significant antibodies (IgG), an important reason to use neutralization techniques**
Potentiators

• Potentiators are usually used in both antibody screening and identification to increase the speed and sensitivity of the antibody attachment to the red cell antigen

• Enhancement medium - Bovine serum albumins, Proteolytic enzymes, Low Ionic Strength Salt Solution, Poly Ethlyene Glycol

• Potentiators have their own uses as well as limitations
Resolving difficult antibody identification problems

- Multiple antibodies
  1. Extended cell panel
  2. Enzyme techniques
  3. Absorption and elution
  4. Neutralization
• Antibody to High Frequency Antigen

1. Panel cells lacking HFA required
2. Ethnicity of the patient – phenotype common to one population may be rare in other population

   Eg – U antigen
• Antibody to Low Frequency Antigen

  1. Less than 10% of the population
  2. Indicated when antibody screen negative and crossmatch incompatible
  3. Cells positive for low frequency antigens required
  4. Compatible blood easy to find
Autoantibody in patient’s serum

**Warm Autoantibodies**

- ABO typing usually not affected
- Rh typing – Use a low protein anti-D reagent
- A warm autoantibody in patient’s serum may mask the presence of clinically significant alloantibodies

1. Remove red cell bound autoantibody followed by autoabsorption
2. Elute autoantibody
3. Identification of auto and alloantibodies
4. If autoabsorption not possible, allogenic absorptions to be done
Cold Autoantibodies

- May cause discrepancies in ABO testing. Resolve this by keeping the sample at 37°C after collection, wash with 37°C saline.
- May cause false positive Rh reactions. Use low protein reagents like monoclonal blend or chemically modified anti-D.
- Antibody identification.
Thank you...!