Basic Immunohaematology

AIMS NZIMLS South Pacific Congress
Gold Coast
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Exceptional People. Exceptional Care.

Requirements for MLS in Blood Bank

• Blood Grouping & Antibody Screening
  – CAT used primarily – automated systems
• Resolve any discrepancies in Blood Group
• Resolve issues with Antibody Screen
• Identify common antibodies
• Select crossmatch and issue appropriate RBCs in an acceptable timeframe
• Phenotype patient and red cells
• Direct Antiglobulin Test

Basic Immunohaematology

• Expect Scientists to know the basics
  – After 6 months of instruction
• Scientist will work unassisted over 24 hr period
• Challenges
  – Identifying antibodies and providing red cells when patient is bleeding or grossly anaemic – usually associated with pressure
  – Issuing multiple products as well as performing & interpreting coagulation testing following massive transfusion
  – Instructing junior medical staff on test results and interpretation – where to find information
  – Liaising with ARCBS in supply or restocking
  – Using various Computer programmes eg Kestral, BloodNet,
  – Troubleshooting – instrumentation/technique issues.

Basic Immunohaematology

• Introduction – Haemagglutination reaction Methods
• ABO System
• ABO System Anomalies & Discrepancies
• Rh System
• Other Blood Group Systems
• Antibody Screening
• Sources of variation
• Investigative Techniques
Basic Immunohaematology

- Challenge - Unknowns
- Participation
- Feedback

Routine Techniques

Routine IH Techniques

- ABO Grouping
- RhD Grouping
- Antiglobulin Test
  - IAT
  - DAT
- Antibody Screening
ABO Grouping

- Determination of the ABO group is the most critical pretransfusion serologic test
- May be performed in tiles, tubes, MTP, CAT
- Testing should be performed using potent, licensed reagents & according to manufacturer’s instructions

ABO Grouping

- Anti-A, Anti-B and Anti-A/B are used to determine the presence or absence of the appropriate antigens
  - Called the Forward or Cell group
- A₁ and B reagent red cells are used to detect anti-A and anti-B in serum
  - Called the Reverse, Back group or Serum group
- Routine test on donors and patients must include forward and reverse grouping, each serving as check on each other

ABO Grouping Discrepancies

- Occur when the forward and reverse groups do not agree
- ABO grouping is MOST IMPORTANT
- Incorrect grouping could lead to fatality
- Issue group O if in doubt or if an emergency
- Discrepancy can be in either the forward or reverse grouping
- ABO discrepancies should always be resolved!!

RhD Grouping

- Performed using Anti-D blood grouping reagents
- All tests should be performed according to manufacturer’s instructions
- May or may not include the use of a suitable diluent control
- May get false positive results with high protein and patients with positive DAT
RhD Grouping

- Modern reagents are potent, monoclonal, low protein, saline reactive
- Monoclonal IgM anti-D reagents usually produce stronger reactions than polyclonal reagents
- Monoclonals may not detect some qualitative weak D’s
  - depends on clone/s used, check pack insert for specificity details

RhD Grouping

- Demonstration of weak D only required for donor typing
- Some blend reagents require the use of an IAT phase to detect weak D’s
- Care with interpretation – DAT positive especially cord cells

Antibody Screening
• Unexpected antibodies are present in about 2% of the population
• Usually result from known red cell challenge from transfusion or pregnancy
• Screening for the detection of unexpected or atypical blood group antibodies
• Performed by testing the patient’s serum or plasma against a set of unpooled Group O screening cells
• Either a two or three red cell set

Antibody Screening

- AB Screening is designed to detect either IgM or IgG antibodies
- IgA is sometimes implicated in serology
- IgM antibodies are capable of causing direct agglutination
- IgG antibodies usually only sensitise
- Reagents and techniques are used to enhance agglutination including:
  - Solutions containing positively charged molecules
  - Enzymes that modify the red cell surface to reduce zeta potential
  - Low Ionic Strength Solutions (LISS) to reduce zeta potential
  - Bridging antibodies (anti-IgG antibodies)

Antibody Screening

- Detection of unexpected red cell antibodies in patient’s blood is important
- Clinically significant antibodies may cause:
  - Decreased survival of transfused red cells
  - Haemolytic disease of the newborn (HDN)
  - Autoimmune haemolytic anaemia (AIHA)

Antibody Screening

- IAT in tubes using LISS or LISS additives has been the mainstay of antibody screening in the past
- CAT systems are now common
- CAT have a greater simplicity of use – no washing
- Less subjective reading endpoint
- Is more easily automated
- CAT methods may be less useful in antibody investigations
- Tube tests remain an important tool because they are relatively inexpensive, versatile and relatively reliable in experienced hands
- Other techniques employing Albumin, Enzymes, Polybrene and Polyethylene Glycol (PEG) have not gained widespread acceptance
Antibody Screening

- Involves patient’s plasma being tested with red cells of known antigen composition
  - Known as the antibody screening cells
- Antibodies if present in the plasma will react with the screen cells
- Technique used must be able to demonstrate clinically significant antibodies (choice of enhancement)
  - Avoid detection of nuisance antibodies
- Sensitivity and speed

Antibody Screening

2.1.2.1 Pretransfusion testing must include an antibody screen capable of detecting potentially clinically significant red cell antibodies. Clinically significant antibodies are generally those, which are reactive in the indirect antiglobulin test [IAT] performed at 37°C. However, anti-A, -B and -A,B must always be regarded as clinically significant.

2.1.2.2 The antibody screen must be capable of detecting anti-D at a concentration of 0.1 IU/mL or lower.

2.1.2.3 Column agglutination technology [CAT], liquid-phase and solid-phase methods are all suitable for IAT testing. However careful consideration should be given to which method is chosen, as there are differences in the robustness or reliability of each.

2.1.2.4 Alternative methods such as enzyme techniques or Polybrene® methods may be used in addition to [but not instead of] an IAT technique, if the method has been appropriately validated and documented. However these alternative methods may be inferior to the IAT for some clinically significant antibodies.

2.1.2.5 The reagent red cells used for screening must come from at least two separate group O donors and between them express the following antigens: C, c, D, E, e, M, N, S, s, K, k, Fy(a), Fy(b), Jka, Jkb and Lea. The cells from different donors must not be pooled to achieve the desired range of antigen expression.

2.1.2.6 One cell should be of R1R1 and another of R2R2 phenotype.

2.1.2.7 The following phenotypes must be represented in the screening cells: Jka(a+b+), Jka(a+b-), Fya(a+b+), Fya(a+b-); SS and ss phenotypes are also desirable. The higher sensitivity from using reagent red cells with double-dose antigen expression is particularly important for the prevention of delayed transfusion reactions, especially those due to Kidd antibodies.

2.1.2.8 Anti-Kpa and anti-CW are rarely of clinical significance, consequently Kpa(+) and CW(+) screening cells are not essential.
Scoring Reactions

- Results should always be expressed in quantitative terms
- Variation of the degree of agglutination may provide indication of:
  - A mixture of antibodies being present
  - Single antibody showing dosage
  - Antibody reacting at varying strengths due to difference in antigen expression on red cells

Antibody Screening

Interpretation

Negative Antibody Screen
- Provides a high degree of confidence that the patient’s plasma is free of unexpected antibodies

Positive Antibody Screen
- Indicates the presence of a red cell antibody
- An antibody investigation is necessary to identify the specificity of the antibody
- The antibody screen does not identify the antibody

Limitations

- Designed to detect commonly encountered blood group antibodies
- Does not detect antibodies to antigens with low frequencies
  - e.g. C\textsuperscript{+}, Kp\textsuperscript{*}, Lu\textsuperscript{+}, Co\textsuperscript{h}
  - As these antigens are usually not present on the antibody screening cells
- Negative screen may be due to sub-detectable level of antibody
  - No previous transfusion history (unaware of presence of antibody)
  - Patient will be transfused with donor units carrying corresponding antigen
  - May lead to a delayed haemolytic transfusion reaction

Antiglobulin Test

- Used to detect red cells coated with IgG antibodies and/or complement (C\textsubscript{3d})
- AHG reagents are needed for the detection of IgG antibodies because IgG is too small to directly agglutinate red cells
Antiglobulin Test

- Polyspecific AHG contain antibodies to human IgG and the C3d component of complement
- Monospecific AHG contains only one antibody specificity: anti-IgG or anti-C3d
- Anti-IgG should be polyclonal to detect all IgG subtypes.
- Anti-C3d should be monoclonal to prevent reactivity with unwanted C’ (mainly C4).

Antiglobulin Test

- Detects IgG antibodies present in the serum after in vitro adsorption of antibodies onto red cells
- Applications
  - Antibody Screening
  - Compatibility Testing
  - Titration Studies
  - Antibody Identification
  - Red Cell Phenotyping

Direct Antiglobulin Test (DAT)

- Detects in vivo sensitisation of red cells with IgG and/or C3d
- Clinical conditions that may result in a positive DAT:
  - HDN - Haemolytic Disease of the Newborn
  - HTR - Haemolytic Transfusion Reactions
  - AIHA - Autoimmune Haemolytic Anaemia

References

Human Blood Groups
G. Daniels
Modern Blood Banking and Transfusion Practices
DM. Harmening
Blood group Antigen FactsBook
MR. Reid and C. Lomas-Francis
Basic Immunohaematology - Haemagglutination

Immunohaematology

Contents
- Immune response
- Immunoglobulins
- Basic Immunohaematology principles
- Antigen - Antibody Reactions
- Methods for detecting haemagglutination

1. To provide compatible blood transfusions

2. To perform antenatal testing to detect and prevent HDNB

Primary Immune Response

1° Immune Response
- 1st exposure to a foreign antigen
- Lag phase occurs in which no antibody is produced
- Activated B cells are differentiating into plasma cells
- Can be as short as 2-3 days, but maybe weeks or months
- Amount of antibody produced is relatively low
- Over time, antibody level declines to the point where it may be undetectable
- First antibody produced is mainly IgM
- Small amounts of IgG are usually also produced
2° Immune Response
- Second dose of the same antigen is given days or even years later
- Accelerated 2° or anamnestic immune response occurs
- Lag phase is usually very short - > 3 or 4 days
- Due to the presence of memory cells
- Amount of antibody produced rises to a high level
- Antibody level tends to remain high for longer
- Main type of antibody produced is IgG
- Small amounts of IgM are sometimes produced

Immunoglobulins

**IgG**
- 180Å diameter
- Bivalent : 2 antigen binding sites
- Optimum temperature of reaction : 37°C
- Able to cross the placenta
- Some are able to fix complement
- 4 subclasses, determined by structure of heavy chains - IgG 1,2,3,4
- Sensitises red cells
- Principle class of immunoglobulin produced as a result of foreign red cell exposure during pregnancy or transfusion

**IgM**
- 350Å diameter
- Multivalent : theoretically 10 antigen binding sites
- Optimum temperature of reaction : 0 to 4°C (range 0 to 20°C)
- Do not cross the placenta
- Fix complement
- Agglutinate red cells
- May be referred to as “saline-agglutinating antibodies”
- Often non-red cell immune (sometimes referred to as “naturally occurring” antibodies)
**Immunoglobulins**

**IgM**

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**In vivo Antibody Activity**

- Antibodies themselves do not damage cells carrying the corresponding antigens
- Antibodies aid in destruction or removal of antigens from the bloodstream (i.e. incompatible transfused red cells)
- 2 major mechanisms of *in vivo* red cell destruction
  1. Intravascular destruction
     - Red cells are destroyed in the bloodstream due to complement activation
     - Haemoglobin is released into the plasma and urine
  2. Extravascular destruction
     - Red cells sensitised with antibody and/or complement are removed from circulation by cells of the reticulo-endothelial system, primarily by cells in the liver and spleen.

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**Techniques used for *in vitro* Ag/Ab detection:**

- Agglutination
- Precipitation
- Agglutination Inhibition
- Haemolysis
- Other
  - RIA, EIA, FIA and Ab Mediated Cellular Assays

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**Agglutination**

- Occurs if the Ag is particulate eg. rbc
- Major method used in immunoh
- 2 stages
  - 1st Stage: Sensitisation
  - 2nd Stage: Haemagglutination
**Factors Affecting 1st Stage: Sensitisation**

- Affinity Constant of Antibody
- Temperature
- pH
- Incubation Time
- Ionic Strength
- Antigen-Antibody Proportions

**2nd Stage of Agglutination: Cross-linkage**

- Immunoglobulin class
- Distance between rbc
- Additives
- Other factors
- Antiglobulin

**Factors Affecting Haemagglutination Immunoglobulin Class**

- IgG cannot readily crosslink saline suspended rbc
- IgM readily crosslink saline suspended rbc because of their increased size and number of Fab sites

**Factors Affecting Haemagglutination Distance between Red Blood Cells**

- Red cells suspended in saline have a net negative charge at their surface and therefore repel each other.
- The net charge is reflected in the zeta potential; the distance between red cells in an ionic medium is proportional to the zeta potential.
Factors Affecting Haemagglutination
- Additives

When cells suspended in a low ionic strength suspension, the incubation time of cells and antibody containing serum may be reduced due to an increased speed of antibody uptake and increased speed of agglutination lattice formation.

Factors Affecting Haemagglutination
- Antiglobulin

Use of Anti-IgG

- IgG cannot mediate agglutination of saline rbc so, IgG sensitised rbc must be detected by using anti-IgG
- anti-IgG crosslinks rbc by reacting with the Fc portion of 2 IgG molecules present on 2 rbc
- anti-IgG may be IgG or IgM, however needs to react with the Fc portion of human IgG molecules

Haemagglutination Reaction Methods

- Different reaction vessels can be used to demonstrate red cell Ag / Ab reactions
- Each method has its own advantages and disadvantages
  - Tile
  - Test-tube
  - Microplate
- Column Agglutination
  - BioVue CAT
  - Diamed ID MTS
  - Lateral Grifols

Haemagglutination Reaction Methods: Tile methods

- Large flat glass / plastic tile partitioned into small squares
- Require ~15-20% v/v rbc suspension
- RBCs settle forming a layer
- After tilting tile, either haemagglutination or free rbc can be observed
- Suited for direct agglutination
- Is considered a very sensitive method for detection of weak groups
**Haemagglutination Reaction Methods:**

**Tile methods**

- Use 3-5% v/v rbc suspensions
- Tubes are centrifuged, agitated and either rbc dispersal or agglutinates are observed
- Centrifugation allows for closer rbc contact
- **Rapid method**
  - Haemagglutination is as a 3D pellet of rbcs
  - Both saline (direct) and antiglobulin (indirect) phases can be assessed

**Test-Tube methods**

- Use 3-5% v/v rbc suspensions
- Tubes are centrifuged, agitated and either rbc dispersal or agglutinates are observed
- Centrifugation allows for closer rbc contact
- **Rapid method**
  - Haemagglutination is as a 3D pellet of rbcs
  - Both saline (direct) and antiglobulin (indirect) phases can be assessed
Haemagglutination Reaction Methods: Column Agglutination

- Special cards/cassettes with 6 or 8 microtubes
- Usually use 0.8% Cell suspension
- Each microtube consists of a
  - reaction chamber
  - column containing inert particles (beads) which can separate free rbc's from agglutinates
- Commercial systems available
  - BioVue CAT, Diamed IDMTS, Lateral Grifols

Haemagglutination Reaction Methods: Column Agglutination

- Blood groups – Centrifuge
- Antibody Screens
  - Incubation
  - Centrifugation

References

- Human Blood Groups
  - G. Daniels
- Modern Blood Banking and Transfusion Practices
  - DM. Harmening
- Blood group Antigen FactsBook
  - MR. Reid and C. Lomas-Francis
Background — The ABO System

Why most significant?
- ABO Antibodies almost always present
- IgM (fix complement) -
- Rapid intravascular Haemolysis
- High antigen numbers
  - e.g. “A” ag 1 million, “D” antigen 25,000

ABO History

- Dr Karl Landsteiner 1901
- Human blood is classified into types: A, B, AB and O
- The absence of A and B antigens results in potent antibodies directed against the missing antigen. This is peculiar to the ABO blood group system.
- most significant → severe HTR

Thank You

- Craig Cox
- Tim Carroll
Background — The ABO System

- The ABO antigens are not direct genetic products
- Interim step
- Genes code for enzymes (glycosyltransferases) that add specific carbohydrates to the H precursor chain to form the A and B antigens

Antigens of ABO System:

Formation of Blood Group A

Antigens of ABO System:

Blood group B

Antigens of ABO System:

Blood Group AB
Antigens of ABO System:

Blood Group O

Glycolipid

Precursor disaccharide

H

Subgroups:

- Subgroups of A and B
- Believed to be a quantitative and qualitative difference.
- A subgroups more common than B
- A subgroups include A1, A2, A3, Ax.
- 2 main practical points:
  - Subgroups might show weaker reactions with ABO antisera.
  - Group A (weak) subgroups (A2, A3, Ax etc) might develop Anti-A1.

ABO Cell and Serum Group Discrepancies

2 main types
1. Unexpected serum group reactions
   - Most common usually weak or missing anti-A and anti-B
2. Unexpected forward group reactions
   - Weakened or modified A and B ags
*Any discrepancies between forward and reverse group must be investigated & resolved

ABO Discrepancies

1. Unexpected serum group reactions
   - Newborns/ Old Age
   - Hypogammaglobulinaemia (low levels of Abs)
     - AIDS
     - CLL, lymphoma
     - Immunosuppressive drugs
   - Cold Allo or Auto abs IgM - IH Lewis, P,M,N, RhD
   - ABO subgroups (A2, Ax with anti-A1 ?? -- reverse group looks like group O)
   - Others - fibrin, rouleaux..
"cold" Anti-M

Ax with anti-A1

O Pos no or weak reverse gp
"oncology" patient

group A patient weak (no) reverse group: AML on ChemoTx
ABO Discrepancies

2. Unexpected cell group reaction
   - Cord bloods (weak)
   - Cold aggs
   - ABO subgroups
     - \( A_3, \ A_x: \ B_x \) weak - can look like Group O
   - Mixed field (transfusion etc)
   - Acquired B - (Group A changed into a Group AB by bacterial infection)
   - Disease states e.g. leukaemias

Unexpected cell group reaction -

mixed cell populations:
- Recent transfusion of blood of a different ABO group to that of the patient
- Bone marrow transplantation of a different ABO group
- Maternal contamination of a cord specimen
- Foeto-maternal haemorrhage
- Twin to twin transfusion in utero
Acquired B:

- An acquired alteration to the A antigen which results in a B-like structure
- Associated with disease states such as bacterial infection and colon carcinoma
- Caused by de-acetylation of A antigen by enzymes found in *E. coli* and *Clostridium sp*

Resolving Acquired B:

- Check patient diagnosis: Infection?
- Some manufacturers produce anti-B reagent that does not react with acquired B
- Test patients serum with their own RBCs
  - The patients own anti-B will not react with the acquired B antigen on their red cell
    (autologous testing)
ABO Grouping: summary

General considerations:
• ABO Most clinically significant
• System of antigens and antibodies
• Discrepancies must be resolved.
• Repeat the test +/- washed cells
• Check sample condition (lipaemic, haemolysed, clumping)
• Check patient details: age, transfusion history, diagnosis (GI disease, marrow transplant etc)
• Group O emergency.
Partial D Cat VI

Rh System

- 2nd after ABO
- HTRs, HDFN, Warm AIHA
- Rh Antibodies persistent, IgG 37C.
- Variants → confusion?

D antigen has greatest immunogenicity of all blood group antigens. > 80% of D neg will make anti-D if transfused with large vols D +ve blood
- (Kell 5% c 2%)

Rh System

- Should not be called Rhesus - now called Rh
- The most complex Human Blood Group System
- Rh complex - large polypeptide - great variety of ag presentation
- 48 antigens
- main 5 are DCcEe

Rh Antigens

Rh antigens are encoded by 2 highly homologous, essentially adjacent, genes.
Rh Antigens

- CE gene - codes for CE protein. C/c and E/e Cw etc antigens reside on this protein.
- D gene codes for D protein (D Neg = D gene missing).

Rh Antigens

Rh D System terminology

- no standard terminology but common is:
  D Positive - detectable presence of D antigen
  D Negative - No detectable presence of D antigen
  D Variants
    1. Weak D - quantitative
       - weakened expression of D
    2. Partial D qualitative
       - part of D protein missing

D variants

1. Weak D. Quantitative
   - normal D antigen structure but lower than normal numbers of antigen; can be as low as 200 (normal D+ about 25,000 D sites per cell).
   - Whole of D antigen is expressed – all epitopes present.
Rh D System terminology

D variants
2. Partial D. Qualitative

• abnormal D antigen structure (also occ. less than normal numbers)
• called partial D: lack a part of the normal D antigen (pieces missing) and can produce allo-Anti-D
• Due to exchange of genetic material between D and CE genes.

Rh System terminology

Partial Ds
• serologists found different bits missing
• partial Ds have been categorised depending on which bit of normal D antigen is missing
• DII to DVII
• DVI is most common category and most important
• DVI has most missing bits & more likely to produce anti-D
• DVI - different policies donors vs recipients

Partial Ds
Blood Donors (Cord bloods)
Usually tested by most sensitive methods, reagents to detect normal, weak D and all partial D including DVI.

Blood Recipients
Common practice in Australia to test patients with reagent that does not detect DVI.

DVI
Summary: Rh system

- Complex blood group system
- Large variety of antigens
- Terminology is fluid
- Commonly D variants classified as D weak and partial D
- Cause of confusion in blood grouping
- DVI most common partial D, can produce anti-D

Summary: Rh system

- Common Rh D typing strategy
  - Donors & cords: use DVI (+) reagent
    • DVI regarded as Rh Positive
  - Patients: use DVI (-) reagent.
    • DVI regarded as Negative
    • Weak <+ or discrepant: classify as Rh Neg (esp women C.B.Age)
Other Blood group systems:

Nomenclature blood groups systems:
- First blood groups single letter
- More discoveries, abbreviations eg Fy, Jk
- Superscript to designate related ags
- 1961 numeric scheme proposed (Allan and Rosenfield)
- ISBT now updated numerical scheme

Blood groups - cont

- Most blood group system antigens are integral parts of the RBC membrane
- Some antigens are simply adsorbed onto the membrane from surrounding plasma eg. Lewis

Kell Blood group system:

- First new group after development of antiglobulin test - a case of HDNB
- System comprises 22 antigens
- Some of antigens have a distinct racial prevalence eg K Northern Europeans, Jsa African descent
- Kell ag on red cells only
Kell - cont

- Depressed phenotype expression – McLeod phenotype - acanthocytes spherocytes
- Null phenotype Ko - normal discocytes
- Expression of Kell ags depends on Kx, on X chromosome
- Ko have increased amounts of Kx antigen

McLeod Phenotype:

- Named after the propositus (first patient)
- Weakened expression of Kell ags
- Absence of a normal XK1 (Kx gene)
- Morphological and functional RBC defects, compensated haemolysis, muscular and neurological defects
- Association with type 11 CGD (another X linked disorder).

Kell Blood Group System:

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<thead>
<tr>
<th>Genotype</th>
<th>Phenotype</th>
<th>Frequency</th>
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<tr>
<td>kk</td>
<td>k or K-</td>
<td>91%</td>
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<tr>
<td>Kk</td>
<td>Kk</td>
<td>8.5%</td>
</tr>
<tr>
<td>KK</td>
<td>K</td>
<td>0.2%</td>
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<tr>
<td>(Chinese and aboriginals have no K antigen)</td>
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KpA, KpB

<table>
<thead>
<tr>
<th>KpA, KpB</th>
<th>Kpa, Kpb</th>
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<table>
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<th>Kpa, Kpb</th>
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<tbody>
<tr>
<td>1%</td>
<td>&lt;1%</td>
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KpA - Penny, KpB - Reutenberg

Jsa, Jsb

<table>
<thead>
<tr>
<th>Jsa, Jsb</th>
<th>Jsa, Jsb</th>
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<tr>
<td>99.9%</td>
<td>&lt;1%</td>
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\(Jsa, Jsb - Sutter, Job - Matthews\)

In black African people 20% have the Jsa antigen, only 1% JsaJsa

Anti-K can cause both HDN and HTR, 20% bind C', usually IgG.

K0 phenotype

- No K, k, Kpa, Kpb, Jsa, Jsb antigens so is K-, k-, Kpa-, Kpb-, Jsa-, Jsb-;
- Occurs in the homozygous state of K0 gene;
- These people, when transfused with blood other than Ko, can produce antibodies to all of the Kell system antigens;
- Unless they are transfused with K0 blood they develop anti-Ku antibody;
- Can be shown that all bloods except K0 have the Ku antigen i.e. a re Ku+;
- K0 also lack most other antigens of the K system, but do have an antigen called Kx;
- This suggests that Kx antigen may be a basic precursor substance for all the Kell antigens. It is present in trace amounts on all normal kell phenotypes and in larger amounts on neutrophils and monocytes;
- KL antigen is lacking on these cells - KL is found on all other normal kell phenotypes - probably produced from Kx i.e. Kx is a precursor of KL.
**McLeod phenotype**

- at first appeared to be a K0, but found to have weak expressions of k, Kpb and Jsb are very hard to detect, because they are so weak;
- is thought that persons of this phenotype have their Kell system genes controlled by another locus, as their parents are always k, Kpb+, Jsb+, the most common Kell type;
- lack the Kx antigen on the red cells;
- red cells show acanthocytosis and these people show extravascular haemolysis resulting in a chronic, compensated haemolytic anaemia;
- evidence of a functional link for blood group antigens, as there is a link to a disease state, chronic granulomatous disease.

**Duffy blood group system**

**Named after a multiply transfused haemophiliac whose serum contained first detected example of anti-Fya**

- racial differences
- Majority of blacks Fy(a-b-), this is rare in whites
- The absence of Duffy ags on rbc's results in resistance to invasion by *Plasmodium vivax*
- example of selective advantage conferred by a blood group phenotype

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**relationship between McLeod and CGD**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>RBC-Kx</th>
<th>WBC-Kx</th>
<th>KELL-Ag's</th>
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<tr>
<td>Normal</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
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<tr>
<td>Type I CGD</td>
<td>yes</td>
<td>NIL</td>
<td>yes</td>
</tr>
<tr>
<td>Type II CGD</td>
<td>NIL</td>
<td>NIL</td>
<td>weak</td>
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<tr>
<td>McLeod (anti-Kx)</td>
<td>NIL</td>
<td>yes</td>
<td>weak</td>
</tr>
<tr>
<td>K0</td>
<td>incr.</td>
<td>yes</td>
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**Duffy blood group system**

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<thead>
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<th>Genotype</th>
<th>Phenotype</th>
<th>Frequency</th>
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<td>Fya</td>
<td>Fya Fya</td>
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<td>Fya Fyb</td>
<td>Fya Fyb</td>
<td>48%</td>
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<tr>
<td>Fyb</td>
<td>Fyb Fyb</td>
<td>34%</td>
</tr>
<tr>
<td>Fy</td>
<td>---</td>
<td>&lt; 1%</td>
</tr>
</tbody>
</table>

(68% Blacks FyFy - also have no Fy3 or Fy5 antigen but have Fy4 - are resistant to *P. vivax*)

(Fya present in 66% of Caucasians & Fyb in 83%)
(Most Asians are Fya+)

- Anti-Fya and anti-Fyb usually IgG abs produced after tx or pregnancy.
- Enzymes destroy ags.
- Often bind C'
Kidd blood group system

- Mrs Kidd, her son had HDNB (1951)
- Jka and Jkb
- 1959 Jk(a-b-) phenotype discovered, Filipina, DHTM
- Jk(a-b-) Polynesia about 1%
- Cells that have Jka and/or Jkb also have Jk3.

<table>
<thead>
<tr>
<th>Kidd blood group system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
</tr>
<tr>
<td>Jka</td>
</tr>
<tr>
<td>Jka Jkb</td>
</tr>
<tr>
<td>Jkb</td>
</tr>
</tbody>
</table>

(Jka found in 90% of blacks and only 15% of Chinese)
(JkJk reported in Polynesian and Chinese - cells very resistant to lysis in 2M urea - significance)

- Kidd abs typically react poorly in vitro but may result in severe in vivo cell destruction.
- Show dosage - need homozygous screening cells.
- Most are IgG, titres rapidly fall off hence often associated with DHTR.

MNS
Second system to be discovered (1927)
- 43 antigens in system
- Antigens located on glycoproteins
- Antigens on red cells predominantly
- anti-M common, anti-N rare
- anti-M rarely associated with haemolysis
- anti-S, anti-s both HTR and HDNB

<table>
<thead>
<tr>
<th>MNSs Blood Group System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
</tr>
<tr>
<td>MM</td>
</tr>
<tr>
<td>MN</td>
</tr>
<tr>
<td>NN</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Phenotype</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>SS</td>
<td>s</td>
<td>11%</td>
</tr>
<tr>
<td>Ss</td>
<td>Ss</td>
<td>44%</td>
</tr>
<tr>
<td>s</td>
<td>ss</td>
<td>45%</td>
</tr>
</tbody>
</table>

- Anti-M, NRCS Ab often, if react at 37 degree C or in IAHG test clinically significant. Shows dosage.
- Anti-N rare. Anti-S and anti-s also rare, but clinically significant.
**P blood group system:**

- Most of the abs involving P system are cold reactive, IgM
- The Donath-Landsteiner ab, a biphasic haemolysin has P specificity. Antibody attaches in cold, haemolysis when warmed.
- Individuals with parasitic infections may have an anti-P1

---

**P Blood Group System:**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotype</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>P1 and P</td>
<td>80%(Tja+)</td>
</tr>
<tr>
<td>P2</td>
<td>P antigens</td>
<td>20%(Tja+)</td>
</tr>
<tr>
<td>P1Pk</td>
<td>P1 and Pk</td>
<td>&lt;.1%(Tja+)</td>
</tr>
<tr>
<td>P2k</td>
<td>Pk antigen</td>
<td>&lt;.1%(Tja+)</td>
</tr>
<tr>
<td>p</td>
<td>p antigen</td>
<td>&lt;.1%(no P.P1.Pk or Tja)</td>
</tr>
</tbody>
</table>

- Anti-P1 commonly encountered, is an IgM cold reactive agglutinin.
- Usually does not react above room temp (if it does could be significant)

---

**Ii Blood Group System:**

- Adults: I antigen present on red cells of 100% of adults.
- Children: i antigen present on red cells of 100% of newborn children.
- Antigens
  - I and i are found on virtually all red cells;
  - at birth the big I antigen is not found;
  - cord cells are I- and i-;
  - in the first 18 months the i antigen disappears to varying degrees and the I antigen develops;
  - most adult cells appear I+ and i-;
  - Anti-I and anti-i are often found as IgM cold agglutinins.
  - Anti-I found in association with cold agglutin disease. Potent autoanti-I may require use of blood warmers for tx.
  - Anti-i found in some cases of IM, transient haemolytic anaemia

---

**Lutheran blood group system:**

- First described in a patient named Luteran 1945
- 18 antigens
- Lu(a-b-) rare, Lu null
- Main ags Lua (Lu1) Lub (Lu2)

---

Dr Polly Crawford - a blood banker, first person who was discovered to be Lu null
### Lutheran blood group system

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotype</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lua</td>
<td>Lua Lua</td>
<td>&lt;.15%</td>
</tr>
<tr>
<td>Lua Lub</td>
<td>Lua Lub</td>
<td>7.5%</td>
</tr>
<tr>
<td>Lub</td>
<td>Lub Lub</td>
<td>92.5%</td>
</tr>
<tr>
<td>LuLu</td>
<td>Lua-b-</td>
<td>&lt;.01% (lack Lu3)</td>
</tr>
<tr>
<td>InLu (&quot;Dominant&quot;)</td>
<td>Lua-b-</td>
<td>&lt;.01% (traces of Lu3 and others)</td>
</tr>
</tbody>
</table>

- Abs to Lua rare, may agglutinate cells directly even if IgG.
- Anti-Lub rare, usually detected only in IAHG. Best to treat as clinically significant.

### SECRETOR System:

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotype</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>SeSe or Sese</td>
<td>ABH Secretor</td>
<td>77%</td>
</tr>
<tr>
<td>sese</td>
<td>Non-Secretor</td>
<td>23%</td>
</tr>
</tbody>
</table>

The term secretor refers only to the ability of a person to secrete water soluble ABO substances. It has no connection with the secretion of other Blood Group Substances such as LEWIS substance which may also be present in saliva and other secretions.

Secretors secrete antigenic substances dependant on their blood group:
- Saliva of group O secretors has H antigens;
- Saliva of group A secretors has A and H antigens;
- Saliva of group B secretors has B and H antigens;
- Saliva of group AB secretors has A and B and H antigens;
- Saliva of Non-secretors - no A, B or H substances present.

### Lewis Blood group system:

<table>
<thead>
<tr>
<th>Genes present</th>
<th>Phenotype</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Le sese H</td>
<td>Le(a+b-)</td>
<td>22%</td>
</tr>
<tr>
<td>Le Se H</td>
<td>Le(a-b+)</td>
<td>72%</td>
</tr>
<tr>
<td>iLe Se H</td>
<td>Le(a-b-)</td>
<td>6%</td>
</tr>
</tbody>
</table>

### Lewis blood group system:

- Lewis system abs frequently encountered in pre-tx and ante-natal testing
- anti-Lea NRCS, IgM
- anti-Leb two forms - one reacts with cells of A2 and O type anti-LebH, the other anti-Leb reacts with all Le(b+) cells
- antigens CHO carried on proteins or lipids, produced by tissue cells, red cells adsorb
- phenotypes arise from interaction of Lewis and secretor, and H
Lewis blood group system - cont:

- Ags not intrinsic to RBC membrane
- RBCs acquire ags by adsorption
- phenotype determined by interaction of 
  \textit{Lele}, \textit{Sese} and \textit{Hh}
- All \text{Le(a+b-)} are non secretors of ABH
- \text{Le(a-b+)} are ABH secretors
- If group A1 get ALeb, group B BLeb

Lewis blood group system - cont:

- New born infants \text{Le(a-b-)}
- Pregnant women decrease in expression of Lewis ags - can develop anti-Lewis abs
- anti-Lea most common. Seen in \text{Le(a-b-)} phenotypes (NOT \text{Le(a-b+)}), usually IgM. If reacting at 37 C and by IAHG care.
- Anti-Leb, seen in \text{Le(a-b-)} and less often in \text{Le(a+b-)} phenotypes

\textbf{ISBT Human Blood Group Systems}

<table>
<thead>
<tr>
<th>ISBT Number</th>
<th>Name</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>001</td>
<td>ABO</td>
<td>ABO</td>
</tr>
<tr>
<td>002</td>
<td>MNS</td>
<td>MNS</td>
</tr>
<tr>
<td>003</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>004</td>
<td>Rh</td>
<td>RH</td>
</tr>
<tr>
<td>005</td>
<td>Lutheran</td>
<td>LU</td>
</tr>
<tr>
<td>006</td>
<td>Kidd</td>
<td>JK</td>
</tr>
<tr>
<td>007</td>
<td>Lewis</td>
<td>LE</td>
</tr>
<tr>
<td>008</td>
<td>Duffy</td>
<td>FY</td>
</tr>
<tr>
<td>009</td>
<td>Duffy</td>
<td>JF</td>
</tr>
<tr>
<td>010</td>
<td>Diego</td>
<td>DO</td>
</tr>
<tr>
<td>011</td>
<td>Lutheran</td>
<td>LU</td>
</tr>
<tr>
<td>012</td>
<td>Soret</td>
<td>SOR</td>
</tr>
<tr>
<td>013</td>
<td>Stankevich</td>
<td>ST</td>
</tr>
<tr>
<td>014</td>
<td>Landsteiner-Waterman Lw</td>
<td>LW</td>
</tr>
<tr>
<td>015</td>
<td>Chabot-Hawkins</td>
<td>CH-HW</td>
</tr>
<tr>
<td>016</td>
<td>K</td>
<td>K</td>
</tr>
<tr>
<td>017</td>
<td>Gerbich</td>
<td>GE</td>
</tr>
<tr>
<td>018</td>
<td>Cromer</td>
<td>Cres</td>
</tr>
<tr>
<td>019</td>
<td>Colton</td>
<td>CO</td>
</tr>
<tr>
<td>020</td>
<td>Landsteiner</td>
<td>LW</td>
</tr>
<tr>
<td>021</td>
<td>Chido/Rodgers</td>
<td>CH/RG</td>
</tr>
<tr>
<td>022</td>
<td>Dombrock</td>
<td>DO</td>
</tr>
<tr>
<td>023</td>
<td>Raph</td>
<td>RAPH</td>
</tr>
<tr>
<td>024</td>
<td>JMH</td>
<td>JMH</td>
</tr>
</tbody>
</table>

\textbf{Investigative techniques in IH Laboratories}
Antibody Investigation and Identification

- Patient’s plasma is tested against a panel of phenotyped red cells
- Panel usually consists of 11 red cells
- Antibody identification panels are commercially prepared

Features of an Antibody Investigation Cell Panel

- Group O cells from individual donors
- Phenotyped for the most commonly encountered blood group antigens
  - eg. Rh, K, FY, Jk, MNS, P, Le, Lu and Co
- Ideally have homozygous expression for Rh, FY, Jk, Ss antigens
- Detection and identification of clinically significant antibodies is essential

Antibody ID

Autologous Control
- Performed in parallel with antibody screen
  - involves testing patient’s plasma with patient’s red cells
- Positive result indicates the presence of:
  - an autoantibody
  - does not preclude the presence of an alloantibody
  - positive Direct Antiglobulin Test (patient’s rbc already sensitised in vivo)

Antibody Investigation: Steps

- Use of the ID panel
- Tested using the IAT method
- Record reactions on panel sheet
- Record batch no. of panel being used
- Add in screening cell data
- Transcribe the result of the screen cells onto panel sheet
Antibody Investigation: Identification of Specificity

- Comparing the pattern of results with the antigenic composition of the panel cells
- If auto control is negative, reactions are interpreted by considering the negative results first
- If a red cell suspension fails to be agglutinated by the plasma, then the antibody present in the plasma cannot be specific against any antigen present on those cells

Antibody Investigation: Aid Sheet

- Identify the cells that did not react with patient’s plasma
- Cross out the antigens on these negative cells (i.e., excluding antibodies to these antigens)
  - cross X for homozygous, cross / for heterozygous
- If after completion of crossing out, only one antigen remains uncrossed, this will be the specificity of the antibody
- Check to see that all the positive cells for this antigen reacted with the plasma
- The plasma may contain antibody(ies) to any or all of the antigens remaining uncrossed

Antibody Investigation: Aid Sheet

- Often there are 2 or 3 antigens remaining uncrossed, the panel should be extended to include cells both negative and positive for each of the antigens so final conclusion can be made (called cell selection)
- Use Other batches of Phenocell and abtectcells or imported panels
- Even expired packs may be used. Most Australian labs can have up to 192 cells available
- Positive conclusive antibody identification with at least 95% probability cannot be made with less than 3 positive and 3 negative reactions
- Additional cells known to be positive or negative for the Ag corresponding to the suspected antibody(ies) must be tested
Antibody Investigation:
Patient phenotyping

- After the antibody specificity has been identified then the patient’s red cells need to be typed for that antigen
- To confirm alloantibody
- Patient’s red cells should lack that antigen
  - i.e., auto control should be negative
  - typing performed using commercial antisera
  - if typing is positive, then antibody to that Ag is unlikely, if negative then presence of that antibody is possible
  - need to use positive and negative controls for the typing
  - positive control must be heterozygous for Ag, negative control must be negative for Ag (these controls assess specific reactivity of antisera)

<table>
<thead>
<tr>
<th>Kpa</th>
<th>Can be excluded on 1 heterozygote</th>
</tr>
</thead>
<tbody>
<tr>
<td>k</td>
<td>To be excluded on 2 homozygous</td>
</tr>
<tr>
<td>K</td>
<td>To be excluded on 2 homozygous but either 1+1 or 2 heterozygous OK</td>
</tr>
<tr>
<td>Cw</td>
<td>Can be excluded on 1 heterozygote (Cannot be excluded if anti-D present)</td>
</tr>
<tr>
<td>e</td>
<td>To be excluded on 2 homozygous</td>
</tr>
<tr>
<td>E</td>
<td>To be excluded on 2 homozygous</td>
</tr>
<tr>
<td>C</td>
<td>To be excluded on 2 homozygous (or 2 heterozygous if Anti-D present)</td>
</tr>
</tbody>
</table>

Part of the Antibody Investigation Aid Sheet
Complex Antibody Investigations

Multiple Antibodies

Presence of multiple antibodies should be considered when one or more of the following occurs:

- observed pattern of reactivity does not fit that of a single antibody
- variations in reaction strengths occur that cannot be explained by antigen dosage
- unexpected reactions are obtained when attempts are made to confirm the specificity of a single antibody
- different panel cells react at different phases or the effect of enzyme treatment of test cell is variable
Difficult antibodies

Presence of difficult antibodies:

- observed pattern of reactivity does not fit that of a single antibody
- Antibody may be reacting only with homozygous cells and appear negative with heterozygous cells
- This is seen with antibodies like anti-M in CAT systems

Multiple Antibody Mixtures

- Identify the cells that did not react with the patient’s plasma i.e. Cells with negative results
- Proceed to rule out or exclude possible antibody specificities (X for homozygous and \ for heterozygous)
- 2 or more antigens remain uncrossed
- Multiple rbc from other panels
- Type patient’s rbc for the corresponding antigens
- Extended red cell antigen typing (phenotyping)
- Acidify serum, longer incubation, more sensitive method (additive)
Autoantibodies encountered during routine blood grouping, screening and identification

- Reacts with patient’s own cells
- Reacts with cells of different Rh phenotypes R1R1, R2R2
  - i.e. All panel and screen cells and patient’s cells react and therefore interfere with identification of alloantibodies
- May mask presence of alloantibodies
- Removal of the autoantibody by adsorption allows for detection and identification of underlying alloantibody

Autoantibodies removal using autologous (patient’s own) red cells

Autoadsorption procedures involve:

- Mixing plasma containing the autoantibody with enzyme-treated Autologous rbc (enz treatment enhances the adsorption of most autoAbs)
  - Plasma to cell ratio (1 vol plasma to >2 vols of adsorbing rbc)
  - Number of adsorptions (x3 or x4)
- Incubate plasma and cell mixture at a temperature that best facilitates formation of Ag/Ab complexes
  - IgM cold reactive autoantibodies use 4°C
  - IgG warm reactive autoantibodies use 37°C
- Centrifuge plasma/rbc mixture to separate adsorbed plasma from rbc
- Test adsorbed plasma against red cell panel to detect presence of alloantibodies

Elution Techniques

- Heat - good for ABO
- Lui freeze - thaw - good for ABO
- Acid - Glycine (Gamma Elu-kit II)
- Citric Acid
- Chloroform/Trichloroethylene
- Ether

Enzyme Techniques

- Papain
- Bromelain
- Ficin
  - Proteolytic enzymes
  - May enhance reaction strength of some antibodies (weak Rh class)
  - Destroys Duffy and MNS antigens
  - Useful to confirm antibodies detected or to remove an antibodies reaction pattern to make the reactions of other antibodies easier to interpret
**DTT**

- Destroys the pentameric structure of IgM - inactivates
- Useful for removing IgM reactions to confirm and clarify presence of IgG antibodies
- Useful for immune anti-A and anti-B titres
- Useful in ABO incompatibility and HDNB investigation

**AET**

- Aminoethylisothiouronium Bromide
- Inactivates antigens of the Kell blood group system
- Must be freshly made
- Requires a pH meter

**SERUM ACIDIFICATION**

- Enhances weak reactions of M,N, Pr and Sd
- Useful when antibody reaction only on homozygous cells
- MAY make heterozygous reactions detectable

**OTHER**

- Longer incubation
- Increase serum cell ratio - 4 drops
- Enhancement choice - PEG, LISS, LISS Additive etc
- Change detection system eg Bg in CAT is usually undetectable in tube
- Dilution - rarely used
It’s all about the best possible medical care

Sources of variation in the Immunohaematology Laboratory

Sources of variation

“Label all tests, reagents and properly – this should be a non-negotiable rule for all laboratory staff.

• Immunohaematology testing is unique in the pathology discipline in that the tests used tend to be manual and the tests performed in hard to label formats like tiles or tubes. Even with the advent of Column Agglutination Technology, tests are still often manually hand labelled. The following general rules for labelling should be followed:
Sources of variation

- Patient samples received by the laboratory should comply with the laboratory's sample labelling policy. In most cases, this should include the patient's full name, date of birth, hospital record number, date and time of collection, and signature of the collector.

Sources of variation

- Reagents made or modified in-house should be labelled with the product identity, date of manufacture or modification, and identity of the operator.
- Test materials such as glass tubes, tiles, and microplates should be labelled in a manner that the identity of the patient is obvious to another staff member. This is often just the laboratory accession number, but this is very important and aids in the prevention of transcription errors.

Other Factors

**Temperature**
- "Get the temperature right as it is vital for test performance."
- Thermal amplitude of antibodies
- Too low / Too high
- RT
- Cooling reagents
- Warm reagents

**Time**
- "Timing matters."
- Sensitivity
- Ab competition
- Enzymes
- Immediate Spin

Other Factors

**Washing**
- Washing cord cells 6 x manually
- PEG

**Reagents**
- "Modern reagents are robust but still must be stored correctly."
- Expiry
- Storage and Transport
- QC
- Each vial should be checked for normal appearance and absence of turbidity.
- Check the product name is correct for the test to be performed and that the product is in date.
- Appropriate controls - Heterozygote phenotype
- Volumes - droppers
Red Cells

“Fresh is best – store and treat Reagent Red Cells carefully and use pooled cells where appropriate.”
• Concentration – variable test sensitivity
• PCV – how to measure (volumetric vs cell counter vs eyeball guess)
• Freshness – antigen stability over time Fy falls off! – LISS reagents crenate cells and change antigen presentation over time - C, Le, Fy
• QC - Each vial should be inspected before use for normal appearance and absence of hemolysis. Check the product name is correct for the test to be performed and that the product is in date.

Frozen cells – freezing damages membranes – thawing may also damage antigens – cryoprotectant needs careful use and removal after thawing as it is high molecular weight.
• Cells used in automation. Probe contamination.
• Pool cells if required – eg titre method cells of the same phenotype may present antigen numbers +/- 100%
• Diluents – follow instructions
• Hypochlorite - S

Controls

“An uncontrolled test is a dangerous test”
• Controls for all reagents must be properly selected.
• Control Reagent Red Blood Cells
• Control software based systems

Diluents

“Wash solutions and buffers are often forgotten but they can cause problems.”
• Water quality – maintenance of RO systems is often poor.
• pH control
• Buffers – buffer capacity vs toxicity – commercial concentrates and solid tablets must be pHed after reconstitution.

Additives

“Routine LISS Additives are critical components in almost all types of modern IAT testing so understanding their attributes and performance is vital in controlling test performance.”
• LISS and LISS additives
• order of addition – LISS and plasma should never be mixed!
  Protein precipitation – residual saline in cells can dramatically affect ionic strength of final incubation solution and therefore test sensitivity.
• PEG – washing - cannot read at 37°C precipitates out
Centrifuges

“Don’t let your centrifuge spin out of control.”

Centrifuges are a vital instrument for tube and CAT testing.

- The centripetal force is measured in Relative Centrifugal Force (RCF).
- RCF has no units but is expressed relative to the earth’s gravitational force in number of gravities (x g).
- RCF is a product of the rotational speed and the radius of the centrifuge.
- Note that the radius is the distance from the centre of the rotor shaft the bottom of the tube when the tube is spinning.
- As most serological centrifuges have swinging buckets, this distance is when the tube is at full horizontal extension.

The RCF may be calculated by:

\[ RCF \ (in \ g) = 0.00001118 \times radius \ of \ rotation \ (cm) \times RPM^2 \]

RPM - Stands for revolutions per minute - the speed you set your centrifuge to spin your rotor.

- High Speed 1000 RCF
- Low Speed 500 RCF
- Voltage
- Calibration Optical vs Vibrational
- Swinging buckets
- Microplate difference in outer
- Timing

Tubes

“Use clean borosilicate glass – never plastic”

- Glass vs plastic
- Size 10 vs 12
- Constitution (glass vs plastic)
- Cleanliness
- Reading
- Tip and roll
- Light sources
- Microscopic reading

Tiles

“While an old fashioned technique, the tile technique can be the most sensitive method to detect weak reactions like ABO subgroups and mixed field reactions.”

- Cleanliness
- Incubation in moist chamber
- Drying
- Type and size
- Disposable
Column Agglutination Technology

“CAT has pros and cons – understand what it can and cannot do”
- Cell load = PCV
- Additives
- Cell diluents
- Incubation time
- Centrifugation
- Reading
- Control
- IgM vs IgG

Column Agglutination Technology - Cell Suspension

Column Agglutination Technology - Cell Suspension

Column Agglutination Technology - Cell Suspension

Column Agglutination Technology - Cell Suspension

Column Agglutination Technology - Cell Suspension

Column Agglutination Technology - Cell Suspension
Reaction reading and grading

- Practice
- AABB scoring descriptions
- Consistency
- External and internal QAP