Compatibility Testing and the indirect antiglobulin test

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What is compatibility?

When donor red cells are transfused they are not destroyed by the recipient’s immune system - they survive normally in the patient.
What is incompatibility?

When the donor red cells are destroyed by the recipient’s immune system - they DO NOT have a near normal life span.

The destruction might be immediate or delayed.
What is the consequence of an incompatible transfusion?

A range of consequences from
• death of the patient [ABO incompatibility]
  to
• the Hb is not increased

The Hb might go up at first but as cells are destroyed it falls again

To benefit the patient the blood must be compatible
Antibodies & incompatibility

Red cell antibodies produced in response to pregnancy or transfusion

Study 1, 1995: 17/319 [5%] non-Tx pts: produced abs. after av 3 units

Study 2, 2005-09: 3002 non-Tx pts: after 5 units 1% pts have antibodies - 2.4% after 10 units
Antibodies produced

• Study 1 anti-E, -K, -Fya, -Jka, -S

• Study 2 anti-E, -K, -Jka, -Cw, -S

In Europe 75% of antibodies are Rh inc. anti-D, -c, mainly from pregnancy
African Studies

Uganda - 6.1% of 214 transfused pts had antibodies, anti-E, -S, -D, -K

Malawi - 1,000 random pts
- 1% had abs, anti-S, -D, -M
- 10% of 1,000 pts previous Tx

Ghana - 886 ab screens, 8 anti-D [1%]
Anti-S

- Anti-S appears to be more common in Africa than Europe
- Causes HTRs & HDN
- Must be detected in XM, reacts by IAT

S neg in Black Africans ~30%
Malawi 36.6%    UK 55%
ABO incompatibility

• ABO compatible is essential.
• ABO incompatibility KILLS
• Naturally occurring anti-A/B found in all but group AB people.
• If group A blood given to a group O adult then that person’s anti-A will destroy the group A cells very quickly.
Why RhD type?

Is the Rh D type important?

If the patient is D negative then a transfusion of D positive blood could stimulate them to produce anti-D.

If D positive blood was then transfused those red cells would be destroyed & the Hb not rise, or cause morbidity.

A transfusion reaction
Right blood to right patient

It is essential the right blood gets to the right patient – wrong blood kills

Therefore care MUST be taken throughout the whole process, from the patient’s sample being taken & labelled, to that patient receiving the correct, compatible units.
Compatibility

Send sample to laboratory with a request form giving the correct details of the patient & the reason for the request, type/volume of component needed & when.

If the patient’s been transfused, a fresh sample is needed if it’s more than 48 hours since last one taken for XM.

Crossmatched blood is normally reserved for only 48 hours after time required.
Selecting the correct product?

To get the desired result the correct blood component must be given

- Whole blood
- RBCs in SAGM or 'packed cells'

To make best use of donated blood select correct volume

- Adult - whole unit 500ml
- Child - paediatric packs ~100 ml
How do we ensure blood is compatible

• We MUST ABO group the patient
• Then select a unit of blood which is of the same ABO group, or is ABO compatible.

Group O patient select group O
Group A patient select group A or O*
Group B patient select group B or O*
Group AB patient select group AB, A*, B*, O*

*in SAGM if possible
How do we ensure blood is compatible

- In D negative pregnant women anti-D could cause their fetus harm – HDFN
- Therefore we need to D type patients and ensure, at least, that D negative females of child bearing potential receive D negative red cells.
How do we ensure blood is compatible

• Therefore we must ABO and D type all potential recipients.
• Select blood that is ABO compatible
• For D negative females, at least, also D negative
• Then perform a CROSSMATCH
The Crossmatch

• The crossmatch is a laboratory test to ensure there is no serological incompatibility between the patient’s serum or plasma & the donor red cells.

• It should detect any major errors in ABO grouping the donor or patient.

Don’t forget it’s ABO that kills
Saline Crossmatch

Saline crossmatch to detect ABO incompatibility

- In a properly labelled tube place 3-4 drops of the patient’s serum [or plasma]
- Add 1 drop of washed 3% suspension of donor cells and MIX
- Centrifuge lightly or leave on bench for 45-60 mins
- Examine for lysis & aglutination
- Either means the blood is INCOMPATIBLE
Crossmatching

• The saline XM will detect ABO antibodies but not other IgG antibodies the patient might have.

• To detect these IgG antibodies perform an *Indirect Antiglobulin Test*, IAT [Coombs Test]
Indirect Antiglobulin Test

• An IAT should be performed for all adults receiving a transfusion or anyone who has been transfused before.

• It is better to perform an IAT XM for ALL red cell transfusions
Safe Blood and Blood Products

Module 3 Blood Group Serology

World Health Organization
Global Programme on AIDS
GENEVA
The IAT XM is usually performed as a ‘one tube XM – immediate spin & IAT’

- 1 drop of 3% washed donor cells
- 3-4 drops of patient’s serum or plasma
- MIX
- Centrifuge lightly
- Look for lysis & agglutination.
- If negative MIX ......
IAT Crossmatch 2

• Place tube at 37°C in a water bath or incubator for 45-60 minutes
• Look for haemolysis & agglutination
• If not present wash cells x3 in clean saline
• Add AHG, mix & centrifuge
• Look for agglutination – if none seen check with sensitised cells
• If the unit is compatible complete your records & label the unit
In an emergency what must be done?
It does depend on how quickly the blood is really needed.
If it is needed NOW – to save a life – select group O [D neg if for female]
Label with patient’s details, record in lab register & issue
Emergency XM

• If you have time - do ABO, D group & select group compatible blood
• Label with patient’s details, record in lab register & issue
• A XM can be set-up & read as normal - if it’s incompatible inform doctor immediately
Urgent XM

- If it’s less urgent do ABO D group, select blood & set up one-tube XM
- Blood can be issued after the spin has been done providing it’s compatible – ABO compatible
- The incubation for the IAT can be reduced to 30 minutes before washing etc
- But where possible do a full XM
Incompatible XM

• If spin phase is incompatible it is probably an ABO error
• Group both the patient’s sample & the donation again
• If an error has been made then select correct blood & re-XM
• If the label on the donation is wrong inform BTS at once
Incompatible XM

- If groups are correct then select more units & XM these
- Try to identify the ab specificity
- If you can not find any compatible inform the patient’s doctor at once
- If blood must be given contact BTS for advice & send sample for investigation
- If necessary issue units that give the weakest reactions in the XM
Quality control of IAT

• Check cell washing procedure

• Add IgG sensitised cells to ALL negative reactions

• Participate in an external quality assessment scheme [EQAS]
Controlling cell washing

• Use at least 4 tubes
• 1 drop of IgG sensitised cells
• 3 drops antibody-free serum
• Wash in normal way [saline pH7]
• Add AHG as per insert
• Spin & read
• Should get a positive reaction
Use of IgG sens cells

It is most important to check that in a negative test the AHG has not been neutralised by inadequate washing. This is done by adding cells coated with IgG antibody – these should be agglutinated by the still active AHG. Only when this check has been done can the result be recorded as a true negative.
Weak antibody control

• Use a weak antibody eg anti-D to control the sensitivity of your IAT

• Set up as for IAT XM with D+ cells

• Should be able to detect 0.1 iu/ml anti-D
EQAS: local schemes

Malawi National Quality Assessment Scheme for Hospital Blood Banks

• Part of national training run by MBTS for HBB staff to improve practice
• HBB staff attend a weeks course
• When trained join NQAS
• x2 group/XM exercises a year
Malawi NQAS-HBB

Malawi NQAS-HBB;
- 2007 14 HBBs,
- 2011 49 HBBs;
- 70% increase in use of IAT in XM
Results returned to hospital Director & lab - scheme taken seriously
But still not 100% get right results
Malawi NQAS-HBB

- 2007 av 70% HBBs correct XM result
- 2011 av 79% got correct XM result
- 99% correct ABO RhD results

- Room for improvement - MBTS still running courses & offering advice
NEQAS

• International scheme - UK NEQAS-Blood Transfusion Laboratory Practice [BTLP]
  4 exercises a year, group, XM

See btlp@ukneqas.org.uk

IgG antibody control for making IgG sensitised cells
contact: reagentscs@nhsbt.nhs.uk
IAT

• Easy for errors to happen
• But with care these should not happen
• Use proper controls
  ....... and you should be able to detect clinically significant antibodies
## Sources of Error in the IAT

<table>
<thead>
<tr>
<th>False Positive</th>
<th>False Negative</th>
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<tbody>
<tr>
<td>• Particles in tubes</td>
<td>• Inadequate washing</td>
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<tr>
<td>• Poor washing of tubes</td>
<td>• Neutralisation of AHG</td>
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<tr>
<td>• Clots</td>
<td>• Under / over centrifugation</td>
</tr>
<tr>
<td>• Over centrifugation</td>
<td>• Short incubation time</td>
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<tr>
<td>• Anticipation of positive result!</td>
<td>• $pH$ of saline</td>
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<tr>
<td></td>
<td>• Clots</td>
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<td>• Failure to add AHG</td>
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<td>• Inadequate volume of AHG</td>
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<td></td>
<td>• Wrong serum to cell ratio</td>
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<td>• Cell suspension too strong</td>
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<td>• Over vigorous shaking</td>
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<td></td>
<td>• Incomplete decanting of wash saline</td>
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<tr>
<td></td>
<td>• Leaving too long between washing and adding AHG</td>
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<td>• Too long between adding AHG and reading</td>
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Thank you for listening

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IgG antibody control
for making IgG sensitised cells contact:
reagentscs@nhsbt.nhs.uk