BLOOD TRANSFUSION

Additional notes to " Immunology notes " -
http://www.scribd.com/doc/11855527/immunology-notes

#1. BLOOD COMPONENTS

Reason for using components
Smaller volume; higher potency
Longer storage life
Specific properties

vs. Whole blood like 'shotgun therapy'.

Require: knowledge of need of patient
knowledge of component

Cellular blood components
Whole blood
Red Blood Cells
leukocyte-poor RBC
platelets
Leukocytes (WBC)

Plasma components
Liquid plasma
Dried plasma
FFP
Cryoprecipitate
VI AHF (Virally Inactivated Anti-hemolytic Factor)
Factor IX
Albumin
Stabilized Human Serum
Immunoglobulin

WHOLE BLOOD
Fresh whole blood
Indications
Open heart surgery (hypovolaemic treatment)
Exchange transfusions
Storage: 2-10 deg. Celsius
Expiry: 96 hours
Whole blood (> 96 hrs.)
Indications
Massive trauma
Acute hemorrhage
Replacement after burns
Established hypovolaemic shock
Storage: 35 days in CPDA-1 preservative @ 2-10 Celsius
Notes
Issue packed cells and colloid solutions if whole blood not available.
Monitor hemostasis.
Do not add drugs or Ca to drip

Inform blood bank if dextran or HES used (interferes with crossmatch)

RED CELL CONCENTRATES

Procedure

Whole blood collected from donor in CPD (Citrate Phosphate Dextrose)
spin @ 2500g for 30 min. @ 4 Celsius
remove 290 ml supernatant plasma
seal
Add SAGM preservative to RBC (110 ml)
Hematocrit = 60%

Indications

Correct a RBC deficit
Signs of O2 deprivation
Replacement therapy: increase O2 and Hb without overall increase in volume (lower risk of circulatory overload).
Lower risk of HLA antibodies reacting to WBC in product.
Non-iron deficient anaemia, eg. Hemolytic anaemia.
Continued hemorrhage
Storage: 35 days in SAG-M preservative @ 2-10 C

Notes

Add no fluid, drugs or Ca to drip.
No saline addition necessary.
Bone marrow patients need leukocyte-poor RBC.
Intra-uterine transfusions need leukocyte-poor RBC.

Leukocyte-poor RBC

Ongoing research.
Still HLA reactions.
Any transfused WBC may cause graft-vs-host disease in immunosuppressed patients.

3 types of Leukocyte-poor RBC

1.Washed RBC

washed with saline
80% WBC removal
all plasma removed
takes +_ 4 hrs to prepare.

Indications

HLA reaction history
Allergic reactions
IgA deficient
Coagulation factor inhibitors
BM patients
Intra-uterine transfusions
Storage: use within 24 hrs

2.Frozen Stored RBC

Made with whole blood < 10 days old.
Plasma removed.
Added = volume glycerol
plastic freezing bag stored in N vapor @ - 150 Celsius
Thawed before use @ 45 Celsius, agitation.
Washed x1 3% saline, x2 0.9% saline. Last wash should have no free hemolysis.
98% WBC removal
30% RBC loss.

Indications
History of HLA reaction.
Long term therapy (HLA sensitization may develop).
Storage of rare phenotypes.
Storage: 7 years @ minus 150 deg.C.
Infuse within 24 hrs of thawing.
3.Leucocyte Filtered RBC
Plasma removed within 6 hrs
Filtered as requested. Takes 1 hr to prepare. Eg. Pall filters.
RBC and platelets pass through filter. WBC absorbed.
Indications
History of repeated WBC reaction for long-term patients.
Prevention of HLA immunization in long-term patients.
Prospective transplant patients.
Prevention of CM-Virus transmission.
Storage: use < 7days old blood. Issue ASAP after filtering.
Risk: pack is opened (closed system breached) to insert filter.

PLATELETS
Control bleeding.
Chemotherapy patients.
NB manage and monitor patient.
Establish thrombocytopaenia, eg immune thrombocytopaenia > low platelet survival.
General uses:
Defective platelet production (aplastic anemia, leukemia).
Platelet dilution (massive transfusions).
Defective platelets (congenital disorders).
Consumption (DIC). http://en.wikipedia.org/wiki/Disseminated_intravascular_coagulation
Evidence of low platelets:
Oozing from stitches.
Oozing from venipunctures.
Petichiae.
Bleeding under skin.
Counts: < 5 x 10^9 / L = life threatening
20 – 50 x 10^9 / L = bleeding with trauma
150 x 10^9 / L = rare bleeding.

Random donor platelets
Pooled platelets.
Prepared within 48 hrs of collection.
Indications:
Major surgery
trauma requiring many units of platelets
post cardiac bypass.
Storage: 5 days @ 22 deg.C., agitation with new plasticizer-free bag.

Apheresis single donor
time consuming (2 hrs), uncomfortable for donor.
Usage:
Long term support: same HLA antigens all the time.
TTD
eg. aplastic anemia, leukemia, anti-platelet ab patients.

Storage: 24 hrs @ 22 deg.C with agitation.
Aheresis platelet-donor must be ABO and Rh match.

LEUKOCYTE CONCENTRATES
Somewhat controversial.
Fresh random units with HES
or single donor apheresis
pre-donor steroid medication > higher yield.
Indications:
Supportive neutropenic patients with gram-negative septicemia not responsive to antibiotics.
Acute viral hemorrhagic fever eg. Congo Fever.
Note:
must be ABO and Rh compatible.
GVHD in severely immuno-compromised patient.

PLASMA PRODUCTS

FFP (Fresh Frozen Plasma)
Prepared within 6 hrs of donation.
Centrifuged, 250 ml separated, frozen @ -18 - -30 deg.C.
Contains all coagulation factors.
Indications:
Replacement of single clotting factor deficiencies if no concentrates available.
Burns
Warfarin reversal before surgery
Vitamin K deficiency with bleeding
DIC
Inherited deficiencies of coagulation inhibitors
Angioneurotic edema
also:
Massive transfusion
Liver disease (Liver source of clotting factors)
Bypass surgery if coagulation disorder evident.
Storage: -23 deg.C. For 1 year.
Thaw and issue within 2 hrs.
Do not re-freeze.
Note:
Thaw between 30-38 deg.C.
ABO compatible; if not, use AB plasma (contains no red cell antibodies)
Rapid infusion.
Not for use as volume expander, unless clotting factor deficiency.

Liquid plasma (not frozen)
Indications for use:
Volume expander
Burns
Shock treatment while waiting for crossmatch
Not for use for clotting factor replacement.
Storage: 2-10 deg.C. For 6 weeks.
-18 deg.C. For 3 years.

Dried plasma
Single donor, <21 day old blood.
High vitamin K concentration. No coagulation factors.
250 ml plasma removed into vacuum bottle, placed on rollers, frozen in thin, even layer @ -60—-70 deg.C., freeze-dried under vacuum.
Indications:
Emergency volume expander.
Protein replacement after burns.
Storage: shelf temp. (below 30 deg.C.) for 7 years.
Reconstitute with 250 ml distilled H2O.

Cryoprecipitate (wet cryo)
Cold insoluble protein left behind after FFP has been thawed.
Snap-frozen 15 minutes @ -70 deg.C.
NB: contains > 50% factor VIII
> 40% fibrinogen
in a volume of 10-15 ml.
Used for intensive therapy without risking hypervolaemia.
Indications:
Hemophilia A
Von Willebrands Disease
Hypofibrinogenaemia

Storage: -20 deg.C. For 6 months.
Thaw @ 37 deg.C for 10 minutes.
Pool 6 bags; use saline to rinse.
Issue ASAP.

Virally Inactivated AHF (Anti-Hemolytic Factor)
Lyophilise (freeze-dry) 3-5 bags of cryoprecipitate.
Indications:
Home treatment of Hemophilia A.
Small children with hemophilia A (small volume of product)
Hemophiliacs needing large doses over long periods eg surgery, severe trauma or major hemorrhages.
Storage: 2-10 deg.C. For 5 years.
Reconstitute to 250 or 500 ml with deionized water.

PROTEIN FRACTIONATION PRODUCTS
Pioneered by Edward Cohn in 1940's.
Principle: selective precipitation of protein eg using change in temp. and pH, eg ethanol precipitation.
- Albumin 20%

method: heat denaturation
Uses:
Shock
Burns
HDN http://en.wikipedia.org/wiki/Hemolytic_disease_of_the_newborn
Hypo-albuminaemia
Use as volume expander.
Storage: 2-10 deg.C. For 3 years.

- Stabilized Human Serum

400 ml plasma; add silica; unwanted proteins precipitate; filter – desalt; heat 56 deg.C. For 3 hrs; subject to UV.

Indications:

Alternative to albumin
Contains all serum proteins (like immunoglobulins and albumin)
Cheaper than albumin
NB in passive immunotherapy
Contains: Immunoglobulins, albumin, transport proteins.
Thus, ideal volume expander.(Also has lower viscosity than plasma)
Storage: 2-10 deg.C. For 2 years.

- Factor IX

Treatment of Hemophilia B.
Storage: 2-10 deg.C. For 2 years.

- Gammaglobulin

Passive immunity to measles, mumps, rubella, pertussis, tetanus, zoster, hepatitis B.
Prophylactic Anti-D.
Storage: 2-10 deg.C. For 3 years.

- Also, PolyGam.

- Fractionated VIAHF (Virally inactivated Anti-hemolytic Factor)

1200 bags of wet cryoprecipitate
remove fibrinogen with buffer
centrifuge
precipitate Factor VIII with another buffer
centrifuged
reconstitute pellet
shell-frozen, lyophilised
heat @ 80 deg.C. For 72 hrs to inactivate viruses.

ADDITIONAL NOTES TO BLOOD PRODUCTS:
HAEMATOLOGY – COAGULATION – Link to follow here.

#2. IMMUNOHAEMATOLOGY

BLOOD GROUP ANTIGENS

Antigenic molecules on blood cells: RBC – called blood factors eg Rh molecules which form an integral part of the RBC membrane, and A and B antigens, and others.

WBC – called HLA (NB role in immune response)

If a foreign blood group is introduced into an individual, an immune response is mounted in an attempt to destroy the foreign protein.

There are 400+ blood group antigens.

GENERAL RULE: **WHATEVER BLOOD GROUP ANTIGENS AN INDIVIDUAL DOES NOT POSESS ON / IN THEIR BLOOD CELLS, THEIR IMMUNE SYSTEM WILL FORM / HAVE ANTIBODIES TO. Eg. In ABO system, patient always have antibodies in their serum to whichever of A and B antigens not on their own Red cells.**

NB: Some blood group antigens are clinically more significant than others.

May result in serious / fatal consequences in medicine.

Definition of an antigen: Protein substance of high molecular weight. If introduced to an individual who lacks it, antibody production is stimulated. The ab will react specifically.

Definition of immunogenecity: (Variability in) capacity to stimulate antibody production.

- different individuals respond different to an antigenic stimulus.
- different antigens vary in their immunogenecity.

Definition of an antibody: Protein produced by the humeral defence system in response to stimulation from an antigen. It will react specifically with that antigen.

Red Cell Antigens

Cell membrane has 3 layers:

2 outer hydrophilic, one inner hydrophobic.

Antigens in all 3 layers. The antigens are products of single genes. Thus, individual-specific.

Homo- or heterozygous http://en.wikipedia.org/wiki/Homozygous

ie double dose (homozyg.) or single dose (heterozyg.). Only sometimes in vitro testing may distinguish double from single dose. Otherwise family studies done.

Definition of an hapten: Molecule which reacts to an antibody, but isn't large enough to stimulate antibody production on its own.

Haem-agglutination

Most Ag/Ab reactions are detected by agglutination.

Definition of haem-agglutination: Process by which antibodies bind RBC together to form clumps.

RBC surrounded by negatively charged field (*zeta potential*). For agglutination to occur, zeta pot. must be: bridged or removed or reduced.

Immunoglobulins

IgM and IgG are the most significant of all blood group antibodies in transfusion.

4 IgG subclasses (IgG1-4) of which IgG1 and IgG3 cover most blood groups.

Complement in immunohaematology

Activated by some ag/ab reactions.

11 components. Labile (deteriorate rapidly).
Ca and Mg essential. Anticoagulants used prevent cpt activation. Thus, fresh serum always used.
Classical pathway:
1 IgM or 2 + IgG molecules needed to activate.
Note IgM (1st response ab) has more binding sites than IgG (2nd response ab).
After binding to cell surface, ab distorts, exposing complement binding site.
Alternative pathway (properdin)
IgA, some IgG
3b component essential
C5 – C9 common pathway.
Cpt system is dynamic: activated, decay, removed, inhibited.

Importance of complement in immunohaematology
NB: If present in ag/ab reaction, *hemolysis* results.
AHG (Coombs test) has anti-cpt to help detect ab binding.
Cpt also used in HLA typing.
Cpt fixation tests.

#3. SEROLOGY

Ag/ab reactions
Ab recognise and combine with ag.
Allow macrophages to engulf and remove it.
Some ab activate complement: process enhanced > hemolysis.
Ab may: agglutinate ag,
hemolyse RBC,
cause sensitization to ag.

Reaction is specific: Ab reacts with one epitope.
Whole molecule reacts.
New product not formed by rection: A+B > AB (not C).
Ag/ab union is firm, but reversible (A and B can be recovered again).
Surface phenomenon: primary structure remain unless complement is activated.
Reactions combine in varying proportions depending on conditions.
Ag/ab reactions very rapid. Combination occurs in 2 stages:
1.Sensitization: ab binds
2.Agglutination (complex becomes visible; visible clumping together of RBCs)
NB to record degree of agglutination – may only be sensitized.
0, 1+, 2+, 3+, 4+
Principles of agglutination
Lock and key: complementarity of shape
Complementarity of charge: opposites attract
Bonding: weak; must be close to be effective.
- ionic bonds between charges of ag and ab.
- H-bonds between proton acceptor and donor.
  V.d.Waals electrons in their orbitals swing to one side > slight positive at one end > nearby negative atom attracted.
  Hydrophobic groups associate in aqueous environment of solution.

- **2 stages: sensitization and agglutination**

1. Sensitization

[ag] + [ab] > [agab] (k1 = forward rate constant)
< (k2 = reverse rate constant)

No loss no gain equilibrium.

$$\frac{[agab]}{[ag][ab]} \quad \frac{k1}{k2}$$

= k (equilibrium constant): reflects strength of bond.

Equilibrium affected by :

- Concentration of Ag and Ab
  If [ag] or [ab] high, then [agab] high.
  NB: If [ag] high (high strength of RBC solution), then [agab] high, but fewer Ab mole per RBC. Thus, agglutination will not be visibly improved.
  If [ab] high (more serum), then [agab] high, with more Ab per RBC > better agglutination of however many RBCs present.
  Thus, sensitivity of blood grouping test is dependant on how many ab's can bind to available RBC (ie.,increased ab > increased reaction strength).
  Thus, higher serum to cell ratio > better sensitivity ... to a point. Too much Ab blocks available Ag binding sites > get *prozone effect* > no agglutination = false negative result.
  Thus, dilute with saline and repeat.
- PH
  best reactivity at pH 6.5-7.5. Some at higher acidity.
- Temp.
  Thermal range of antibodies 4-37 deg.C.
  [agab] formed, the same at lower temp., but rate of reaction is slower.
  eg. anti D takes 20x longer to react at 4 deg.C.
  Therefore, low temp. only used when cold ab (ab,usually IgM which react at low temp.)suspected. Otherwise grouping done at 22-37 deg.C.
- Ionic strength (IS)
  The lower IS, the higher reaction rate.
  Low IS results in lower zeta potential.
  But, if too low, complement also binds and many unwanted positive reactions (*false positives*) occur.
  0.03 I ideal
  LISS (Low IS Solution) > incubation time may be reduced from 30 min. to 10 min.

2. Agglutination

Variables in agglutination: Multiple factors influence agglutination:

Characteristics of Ab
Location and number of Ag sites
Forces holding RBCs apart

Note: should RBC diameter = 50 yards (width of rugby field), then IgG molecules would be bound on surface, spaced 3 inches between combining sites. IgM would be spaced 6 inches.

-Characteristics of Ab

Size and binding sites: IgG or IgM

IgG has 2 binding sites; IgM has 10

Thus, IgM agglutinate in saline (complete ab); IgG does not agglutinate -- must add Coombs reagent (IgG incomplete ab). http://en.wikipedia.org/wiki/Coombs_reagent

- -Number of Ag sites available:
  Many A and B sites (ABO grouping)
  react in saline.
- Location of Ag sites:

A and B protrude above RBC surface;
Rh within the membrane.

- Forces holding RBC apart

Zeta potential attracts positive ions.
.: high density of positive charge buildup close to membrane; reduction of zeta potential.
Also surface tension: membrane lipids on outer surface, H2O molecules create surface tension.
Ab's lower surface tension.

Bovine albumin

If ab does not agglutinate in saline, albumin may enhance by lowering zeta potential.
Dielectric constant (measure of ability to dissipate a charge) raised with albumin. Albumin binds to lipids on the membrane.
Note: only suitable for some ab's.

Enzymes

RBC have negative charge due to carboxyl groups of sialic acid being ionized.
Adding enzymes eg: ficin (figs), papain (paw-paw), bromelain (pineapple), remove sialic acid from membrane > decrease zeta potential.
They also remove glycoprotein from surface, making bllod antigens more exposed to ab.
Bromelain is used to expose Rhesus antigen for Rh test.

Dosage

Some, but not all systems show dosage.
If individual is MM (homozygote) the reaction may be stronger than in an MN (heterozygote).
May even be negative with a heterozygous individual.
Some anti-Rh (Rh ab) show dosage.
ABO system does NOT.

Complement fixation

Eg. to detect White cell antigen.
Ag/ab plus fresh complement.
Ag + Ab, incubate @ 37 deg.C.
Add complement.
> lysis
Add blue dye (tripan blue)
Lysed cells take up dye = positive test for that White cell antigen.

Complete Ab

Cause direct agglutination of RBC. eg. IgM

Incomplete Ab

Cause sensitization only. eg. IgG

The Anti-globulin test (Coombs test)

1945 by Coombs.
Human serum (globulin) injected into goat or rabbit > Animal makes anti-human globulin.
Boosters given; animal bled at various intervals. Remove unwanted antibodies by adsorption.
Anti-human globulin remains (IgG + complement).

Coombs test (direct and indirect)
Direct Coombs (DAT = Direct Antiglobulin Test) used to detect antibodies bound to a patient's red cells.
Indirect Coombs (IAT) is used for example to see if a Rh negative mother has Rhesus antibodies in her serum (anti-Rh IgG can cross the placenta > Hemolitic disease of Newborn if baby Rh+).
Known Rh+ RBC incubated with mother's serum. Coombs done to check if the RBC's have been sensitized by mother's (incomplete) antibodies.
Coombs test:
Wash off excess antibodies.
Add Coombs reagent.
Binds to human IgG's bound on RBC. Causes visible clumping = pos. Coombs.

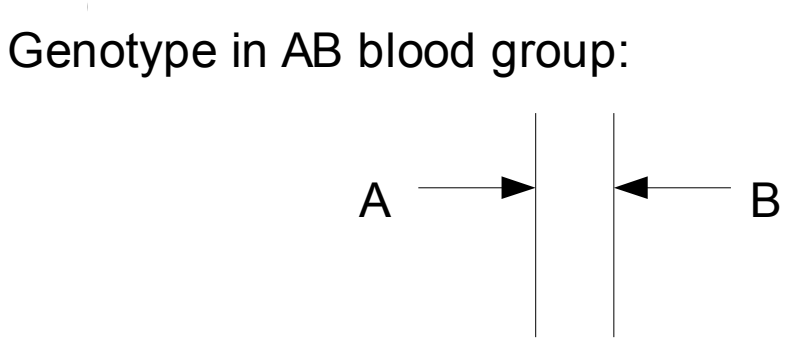
#4. PATIENT PHENOTYPES IN BLOOD TRANSFUSION

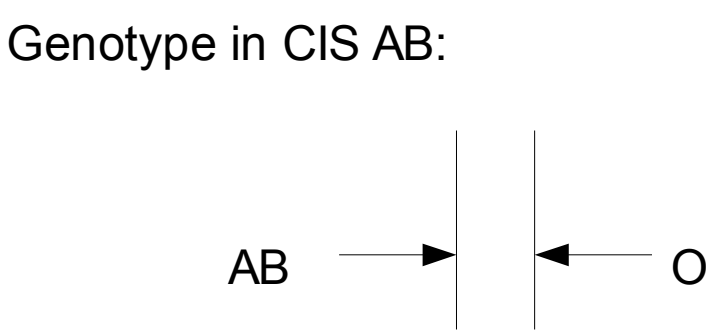
The Bombay Phenotype (Oh)

http://en.wikipedia.org/wiki/Bombay_phenotype

The H-antigen is found on all people's red cells, but lacking in a few individuals who have inherited the recessive gene (h) from both parents, ie. Genotype hh.
Thus, they do not have H-antigen on their red cells.
However, H-antigen is also a building block for the A and B antigens, so they also lack these (even while possessing the genes for A and/or B) hence,Group O.
But, they cannot receive blood from Group O donors (the universal donor, lacking the highly immunogenic A and B antigens on their red cells). Group O donors still have H red cell ag.
Anti-H formed in Bombay patients is powerful and haemolytic.
Only Bombay blood is compatible.
Note; they can still transmit A or B gene to offspring as the genes are normal.

CIS AB
Phenotypic AB.
But genetically rare, since AB together on one allele of the gene pair.





Important for medicolegal reasons ,eg paternity dispute, especially if mother is Group O.

T-antigen activation
In presence of infection, enzyme produced which alters red cell membrane.
An antigen, T is exposed.
All adults have anti-T in their serum.
Such a sample will thus react with all adult serum, giving false +ve in cross-matching.
Not significant except that identification is difficult due to poly-agglutination.

ABH in disease

Leukemia
Strength of A ag varies 4+ to negative.
Thus, clinical info of patient vital.
Saliva normal.
Reverse grouping normal (patient serum remains normal).
Weaker B and H antigens possible.
Ag levels return to normal in remission.

Carcinoma:
Colon, rectum
RBC acquire weak B ag, thus with anti-B a weak pos. reaction formed.
Reverse grouping normal.
Mechanism: possibly colon bacteria eg E.coli and Proteus' B-like antigens leaching into blood.
Stomach, pancreas
Difficult to group.
Plasma contains antigens that neutralise antisera. Cells must be washed first.
Saliva helps grouping.

ABO in transfusion
Most fatal incompatible transfusions are ABO linked.
NB: a teaspoon-full of the wrong ABO Group infused into a patient is sufficient to kill the patient !
Fortunately very rare occurrence.
Most errors not in the lab; rather clerical (eg mislabled specimens)
Major incompatibility: patient has antibodies to donor cells. Major immune response.
Minor incompatibility: donor blood has antibodies to patient ag. Eg. O donor blood has anti-A, -B, -AB. Given to A-patient in emergency (O universal donor). A type of GVHD. Therefore titre done on O donor blood.
Only low titre O donor blood given to other Group patients in emergency.
However, usually packed cells given anyway, so not much donor serum received. Minimizes complications.
Why is major ABO incompatibility so severe ?
Always antibody in patient.
Ab have high titre.
Ab can activate complement. Cpt factors readily available in circulation.
Can cause hemolysis.
Patient body temp. ideal for reaction.

Large amounts of A and B antigens exposed on RBC surface.

The Lewis System
1946 Mourant discovered anti-Le^a
Le antigen is soluble in plasma; adsorbed onto RBC.
Genes:
Alleles: Le and le
Not present on newborn RBC. Develops later.
Genotypes: Le(a+b-)
Le(a-b+)
Le(a-b-)
Determined by: Le/le
H/h
Se/se (secretor gene)
Le gene makes Le^a substance.
If Se and H also inherited, Le^b is made (Le^a converted to Le^b if Le+H+Se present).
No genotype Le(a+b+) exists. But when Le^b is formed, some chains will remain Le^a. So, weak Le^a may be detected with the Le^b.

Genes	RBC phenotype	Secretor ?
Le, H, se	Le(a+b-)	NO
Le, H, Se	Le(a-b+)	YES
le, H, Se	Le(a-b-)	YES
(le recessive > Le^a not made)		
le, H, se	Le(a-b-)	NO

Phenotypes: Le(a+b-) 22% of pop.
Le(a-b+) 72% of pop. (note 80% of pop. have Se)
Le(a-b-) 6% of pop.

Lewis antibodies
20% of Le(a-b-) people have.
More common in certain population groups.
Complete ab (IgM)
Low temp. reactive (22 deg.C.)
Also reacts at 37 deg.C. With Coombs + enzyme.(enzyme to expose hidden ag's)
Anti-Le^a cannot be found in Le(a-b+) individuals, since Le^b comes from Le^a.
Sometimes anti-Le^a and anti-Le^b found in Le(a-b-) patients.
Sometimes only anti-Le^b in Le(a-b-) patients.

Lewis antigens are depressed in pregnant women > may develop anti-Le.
Cord cells are Le(a-b-) till a few weeks.
Adding adult plasma to cord cells can transform them.
Note: anti-Le do not cause HDN.

Transfusion.
If donor is Le(a-) and patient Le(a+), patient's Le(a+) adsorbs onto donor cells. Patient's Le status changes for a period of time.
If donor is Le(a+) and patient is Le(a-), patient's plasma elutes Le(a+) and patient becomes Le(a+).
Anti-Le^b may be hemolytic. If positive ab on crossmatch, repeat at body temp.(37 deg.C.). If negative at that temp., blood can be issued. Lewis ab not active at that temp. (Lewis ab is cold

reacting ab.)

The Ii System
I-Antigens
Also carried on the ABH chains on RBC.
I very close to membrane, so ABH may mask I ag.
Not surprising that Bombay (Oh) cells react strongly with anti-I.
Most adults are I positive.
Few adults are i-positive.
Babies ii, converted to II.
All infants i-pos. Slowly replaced and converted to I (+- 18 months).
I also found in secretions (saliva and breast milk).
Disease
- Abnormal erythropoeisis like aplastic anemia > increased i-antigen.
- Infectious mononucleosis > anti-i
- Alcoholic cirrhosis > anti-i
- Anti-I in patients recovering from mycoplasma pneumonia.

Antibodies
Anti-I:
Cold Ab (IgM). reacts @ RT.
Auto-ab, ie from I-pos. Patients. Note: most adults I-positive.
Anti-I causes grouping problems. All transfusion units incompatible.
Test @ 37 deg.C. (body temp.) usually negative.
Anti-I regularly found in I neg. patients.
No clinical significance.
Anti-i
Rare
Reacts with cord cells.

Lewis system in transfusion:
Neither Le-, nor I/i ab's cause HDN
Incompatible units: test @ 37 deg.C. ?Neg.

The Rhesus System
1940 Landsteiner & Weiner
Rhesus monkey blood injected into rabbits > built up Ab.
This ab was found to react in 85% of human RBC.
Called anti-Rh.
85% pop. Rh positive
15% Rh negative.

In 1934, Levine reported an ab implicated in stillborn.
Was shown to be anti-Rh.
Later, Ab from transfusion reactions and implicated in *hydrops foetalis* also turned out to be Rh-ab.
Anti-Lw was suspected, but turned out not to be anti-Rh.
1943, 3 other related ab's C, D and E.
Genetic theories
Fisher-Race
Fisher – mathematician; Race – serologist.
Theory: 3 closely linked loci for genes: C, D and E. Their alleles are c, d and e.
No d-antigen exists. dd is amorphic combination > Rh negative.
Low crossing over, genes inherited together.

More recently, sub-loci have been discovered.
Weiner
Multiple allelic genes code for an agglutinogen made up of 3 factors.
Rosenfield 1962
Numerical system, eg Rh:1 = D

Rh inheritance
Straight forward Mendelian, or passed on as whole (single) gene, eg Cde , in stead of 3 genes.
Frequency varies between races.
No anti-d exists.
Rh and transfusion.
Used to type C, D, and E.
Now only D is typed. If D is negative, patient is Rh negative.
C and E usually absent if D is absent.
Choices with issuing blood:

Patient	1st choice	2nd choice
Rh positive	Rh positive	Rh negative
Rh negative woman (NB)	Rh negative	---
Rh negative post-menopause woman	Rh negative	Rh positive
Rh negative man	Rh negative	Rh positive
Rh positive baby with Rh neg. HDN	Rh negative (to prevent escalation of reaction to mother's IgG (anti-D) in baby > ↑ bilirubin.)	

The D-antigen
Most NB in HDN http://en.wikipedia.org/wiki/Hemolytic_disease_of_the_newborn
Most NB in Tx after ABO.
Anti-D almost always produced after incompatible Tx.
At 12 weeks Rh positive Fetus develops severe HDN from previously sensitized Rh negative mother's anti-D crossing the placenta.
Anti-D transfused into Rh neg. patient also elicits severe HTR (hemolytic transfusion reaction).
D-antigen gives strong positive with enzyme + Coombs.
May even agglutinate without it.
Structure
Phenotype Rh mosaic: Weiner RhoABCD
If apart is missing, ab to that part may build up. Therefore anti-D sometimes found in an apparently Rh positive individual. AHG may be needed to get positive Rh result from such a weakened Rh-Ag.
Weak Rh-Ag called D^u .
There's no anti-D^u as it is weak ag.
Instead Anti-D forms.
Donor appearing Rh-neg. But the D^u antigen can elicit ab formation in an Rh neg. patient.
Another way D^u is formed:
C is trans to D on gene.
Somehow weakens D ag.
Also get D^u ag.
In this case, no piece is missing,

D^u pos. donors are Rh positive.
D^u positive recipients get Rh negative blood.

Genetic Deletions

Whole Rh complex deleted. Rh(null) Syndrome.
C and E missing
only E missing
C and D missing.
Never, D and E missing together.
Deletion patients produce exotic antibodies.

Compound ag

C + D } or alleles
C + E }
D + E(e) never missing together.

Compound ab

Anti-G from Rh0 individuals.
Anti-Ce
Anti-cE
Anti-f (with e)
Only if in trans position.

Genetic Pathways in Rhesus complex

Precursor genes		CDE genes		CDE antigen
X'rX'r	> add sugar >	CDE	>	CDE antigen (normal)
X^0rX^0r (no precursor forms: genes blocked)	>	CDE	>	No CDE antigens are formed. (Rh NULL)
X^QrX^Qr	> modified >	modified CDE	>	Weak CDE antigens (Rh MOD)
X'rX'r (normal precursor)	> add sugar >	rr (faulty genes)	> Blocked >	No CDE antigens (Rh NULL)

The Kell System

1946 Kell ag (KK)
product of Kell gene.
1949 – k allele: Cellano: Kk

Antigens

3 closely linked loci
K – k (Cellano)
Kp^a – Kp^b
Js^a – Js^b

other associatons of gene pairs – up to 22.

The Ko Phenotype.

Silent allele at Kell locus: K^oK^o (recessive)
no expression of Kell antigens
> ab built up against all Kell = Anti-Ku

The McLeod Phenotype.

1961
weak expressions of Kell
ab present: anti-KL
? seperate gene action KX
Chronic Granulomatous Disease associated with McLeod phenotype.
X-linked recessive allele –> men only
Granulocytes not functioning properly (missing Kx antigen) -- engulf bacteria but cannot kill them.
Hemolytic anaemia associated with McLeod phenotype.
Serological characteristics
Incomplete ab (IgG)
warm (37 deg.C.)
requires AHG
NB: *enzymes* do not improve reaction, because Kell ag already protrudes above membrane (enzymes used to remove protruding antigens to expose those hidden beneath them in RBC membrane).
Clinical significance
Strongly immunogenic
IgG
Cause fairly severe HDN, severe HTR (hemolytic transfusion reaction)
99% pop. k positive (high frequency antigen)
If patient has anti-k, must get donor from Rare Donor file.

The Duffy System

1950 -- Anti-Fy^a reported
1951 -- Anti-Fy^b reported
Mendelian dominant genes:
Fy^a Fy^b
plus silent amorphic allele: Fy

Phenotypes	<	Genotype	note: in phenotype; a not converted to .b as in Lewis.
Fy (a+b-)		Fy^a Fy^a	
Fy (a-b+)		Fy^b Fy^b	
Fy (a+b+)		Fy^a Fy^b	
Fy (a-b-)		Fy Fy	

Duffy and Malaria
Most West-African blacks have Fy Fy genotype ie Fy (a-b-) phenotype.
Plasmodium vivax causes benign Malaria. Cannot invade Fy (a-b-) RBC
Thus, most W-Africans immune to malaria.

Synteny
2 gene loci on same chromosome, eg Duffy and Rh both on chromosome 1, but not linked to each other.

Clinical significance
anti-Fy^a – best detected with AHG

Hemolytic Tx reaction (HTR)
HDN

Anti-Fy^b – very rare
only AHG to detect
HTR
not HDN

Fy3, 4 and 5 also reported.

The MNSs System
Genetics:
1927
M + N are products of allelic genes M and N (MM, NN, MN)
1944: Anti-S
1951: Anti-s
M+N and S+s so close together, they're inherited together
MM homozygotes also have small amounts of N-ag, thus conversion M > N
M + N demonstrate dosage well.(dosage: homozyg. MM strong Ag reaction = double dose, compared to heterozyg. MN)
Many other ag eg. U in most people.
Anti-U in S-s people.
Media
Don't use *Bromelain* (enzyme) in MNS
Ag destroyed by Bromelain > false negative result
Often react only below 37 deg.C., ie in saline.
Clinical significance of Anti-M and Anti-N
Most are IgM
Thus, HDN rare (IgM cannot cross placenta)
However, anti-M has been implicated; anti-N rarely
Anti-N common in renal dialysis patients. ? Formaldehyde (similar structure to N antigen).
Anti-S and Anti-s
less frequent
more significant
Anti-S also reacts below 37 deg.C.
Also mostly IgM
IgG examples can cause HDN
also, anti-U may cause HDN, HTR
Lectins (if you don't know what lectins are, read up at: http://en.wikipedia.org/wiki/Lectins
Vicia graminae has Anti-N specificity

The Kidd System
Genetics
1951 – Jk^a
1953 – Jk^b
alleles co-dominant
Jk (a-b-)-individuals unusual (S.American tribes).
Media
Extremely labile ab – serum must be fresh
enzyme and AHG
hemolytic
Clinical Significance

Seldom HDN
NB Delayed TFR
usually in combination with others
usually IgG; some IgM
strongly hemolytic
Anti-Jk^b usually weaker
Anti-Jk^aJk^b (in Jk(a-b-)-indiv. Has been implicated in reactions with neutrophil site.

The Lutheran System
Genetics
1945 Lu^a
1956 Lu^b
Co-dominant Lu(a+b+)
Linked to secretor (Sese)
Lu(a-b-) also reported
Only 8% Lu(a+b-)
Most people(a-b+)
Anti-Lu IgM
Show mixed field agglutination.
Media
low temp.
Mixed field agglutination
Shows dosage
AHG best; enzyme also.
Clinical significance
Usually IgM
May cause HDN
Anti-Lu^b reported in HTR; therefore tested for in panels

The P System
Inheritance
3 genes: P1 > P1 ag and P ag
P2 > P antigen only (with allele p2)
p > no antigen
P1 dominant over P2 and p
P2 dominant over p

Phenotype.	Genotype
P1	P1P1 or P1P2 or P1p
P2	P2P2 or P2p
p	pp only

P2 often has anti-P1 in serum.

P^k
Associated
If P^k positive; no P specificity
Routine tests: only P1 pos. or P2 neg.
Antibodies
Anti-P1 if P2 patient
P1 is a high frequency antigen (eg in hydatid cyst fluid)
Anti-P if P^k in serum (anti-P1 and P2)

Anti-P + P1 + P^k (called Anti-Tj^a) if pp.
Clinical significance
Anti-P1: IgM
low temp.
No significance
Some IgG cases: 37 deg.C. With AHG.
Anti-Tj^a: usually IgM, low temp.
But, if IgG: 37 deg.C.; hemolytic
Possible cause of abortion.

Other Systems
Xg^a
Sex-linked on x-chromosome
89% female; 67% male.
Hemizygous (only half the amount in males)

Dombrock (Do^a Do^b)

En^a
En^a neg. → sialic acid deficiency; suppressed MN ag.

Cartwright (Yt^a Yt^b)
Yy^a is immunogenic.

Diego (Di^a Di^b)
? HDN in 1955
Ag common in Japanese and S.American tribes.

Scianna (Sc:1 Sc:2)

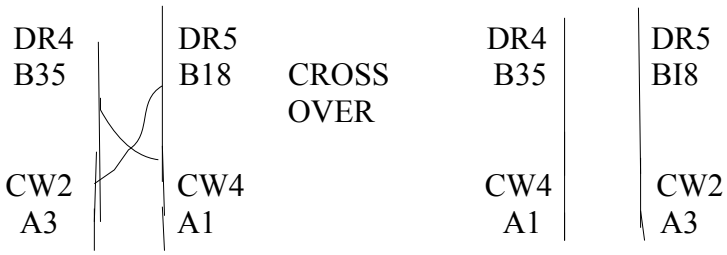
Wright (Wr^a Wr^b)
Ab in auto-immune haemolytic anemia.

#5. WHITE CELL SYSTEMS

The HLA system
Human Leukocyte Antigen
Short arm of chromosome 6
Highly polymorphic
more than 50 loci; some are pseudogenes
3 classes:
Class I – HLA-A
HLA-B
HLA-C
Class II – HLA-DP
HLA-DQ
HLA-DR
Class III – C2
Cpt factor B
C4, and >30 others.

Inheritance
Female phenotype
Class I: A1, A3, B18, B35, CW2, CW4,
Class II: DR4, DR5
Male phenotype
Class I: A2, A11, B7, B35, CW1, CW2,
Class II: DR2, DR3

Child inherits one haplotype (on one chromosome) from each parent,
Eg. A1, B35, CW4, DR4 from mother
for a total of 8 WBC antigens from both parents.
Loci are linked; inherited as a unit.
However, cross-over may occur during mitosis.
While cross-over occurs in HLA system; it does not in Rhesus .
eg. Child:



NEW HAPLOTYPES CREATED
Some haplotypes are common to certain population groups
eg. Caucasians A1 and B8 often found together (linkage disequilibrium)

Clinical applications of HLA testing
Transplantation – donor / patient matching
Disputed paternity testing (DNA fingerprinting more NB now)
HLA disease association
Single donor platelet

- Transplantation

survival of graft better if HLA matches
Blood transfusion if ? Tolerance
Incompatible Bone Marrow tp. > GVHD
D region more NB than A, B or C-locus in matching.

- Paternities

  Comparing man to random man in population.

- Polymorphic haplotype inheritedYet, HLA can give 90 % exclusion
  plus RBC > 95-98% exclusion.

- Disease

Association exists between certain HLA,s and specific disease, eg. HLA-B27 and ankylosing spondylitis. Association means statistically higher incidence; not predictive.

- Apheresis (platelets)

Patients receiving repeated platelet transfusions.
Patients become *refractory* – build up ab's against HLA on platelets.
Thus, HLA match necessary. But, expensive, time consuming; other non-HLA antigens on platelets.

<u>Indications for platelet transfusion</u>
malignancies
platelet deficiency (number and function)
(usually multiple transfusions required; apheresis donor)
Also: severe trauma, cardiac surgery, hemorrhagic fever.(pooled single donor).

<u>Platelet antigens</u>
HLA, ABO, P&I, P1^A1 and P1^A2 (anti-P1^A1 common problem), Yuk^a and Yuk^b, etc.,etc.

#6. IMMUNOHAEMATOLOGY AND DISEASE

HDN

Definition: Hemolytic Disease of the Newborn is a condition where mother's immune system recognizes baby's RBC as foreign.

Sensitisation occurs via: previous pregnancy
blood transfusions

Ab crosses placenta and destroys foetus' RBC.

Outcome varies from mild anaemia to death. Bilirubin from RBC lysis also builds up to toxic levels in baby > brain damage.

Most severe form is anti-D (Rhesus factor).

NB prophylaxis: To prevent sensitization of mother's immune system, administer anti-D to mother after birth, after abortion, after amniocentesis, after turning; if baby is / might be Rh positive.

RhOGam instrumental in combating "Rh disease".

Give within 72 hrs. Attaches to baby-Rh antigens in mother's system, before sensitization can take place.

Kleihauer test: Determines Anti-D dosage required. http://en.wikipedia.org/wiki/Kleihauer_test

NB Always test pregnant woman for: ABO, Rh, Antenatal Screen
http://en.wikipedia.org/wiki/Antenatal_screening

If mother is Rh-negative, monitor for anti-D development.

Test father's Rh. If negative – no problem.

If anti-D identified: advise patient, do titre regularly; if father Rh pos. chances are baby is Rh pos.

If titre rises, HDN likely progressing.

What now ?

26 weeks – clinician options:

May request amniocentesis.

Amniotic fluid optical density measured (for presence of bilirubin). Plotted on Liley chart.

Take at least 2 readings, 2 weeks apart to get trend.

If condition is severe, but it is too early for delivery (26-32 weeks), an intra-uterine transfusion is indicated; perhaps followed 2 weeks later by another.

Plasmapheresis to lower anti-D titre.

32 weeks: if condition severe, do Bubble Test to assess lung maturity (lung surfactant test).

If mature, deliver !

Continuous foetal scans to assess size and ascites.

At birth: Top up transfusion, exchange transfusion, or only phototherapy.

Exchange Tx:

correct anemia

no circulatory overload

remove sensitized cells and replace with normal.

Remove bilirubin

remove maternal ab from baby's circulation.

20 ml aliquots.

AUTO-IMMUNE HAEMOLYTIC DISEASE.

Hemolytic anaemia: shortened lifespan of RBC.

1. Hereditary eg. Haemoglobinopathies http://en.wikipedia.org/wiki/Hemoglobinopathies and sherocytosis.
2. Auto-immune due to auto-antibody.

3. Obscure; secondary to malignancies
4. Drug induced.

AIHA -- Warm reacting Ab
Auto-immune hemolytic anemia – warm ab.
Spleen removes ab/RBC complexes.
Patients have anemia and jaundice (↑ bili.)
Acute hemolytic phase – Hematocrit falls to 5%.
Serology:
Positive Direct Coombs.
Positive Indirect Coombs

IgG and complement coombs positive.

AIHA – Cold reacting Ab
Enhanced reactivity below 37 deg.C.
Transient eg mycoplasma infection.(see also anti-I).
At low enough temperature, hemlysis occurs called Cold Agglutinin Disease.

- Cold Agglutinin Disease

RBC destroyed in distal parts of the body eg fingers and toes.
Low pallor, *acrocyanosis* (blue around lips).
Serology:
Ab with I activity (IgM)
infection caused; recover rapidly.
Idiopathic – benign

- Paroxysmal Cold Haemoglobinuria (PCH)

Rare
Massive hemolysis on exposure to cold.
Donath-Landsteiner antibody.
Also, congenital Syphilis
Abrupt onset; back pain, cramps, fever, haemoglobinuria (Hb in urine).
Serology:
free Hb, bilirubin in blood
DAT (Direct Coombs) positive (with complement)
Donath-Landsteiner ab - anti-P specific.
Patients to be kept warm.

- Obscure haemolytic Anaemia

Malignant complications.
DAT positive.
Jaundice.

- Drug induced Haemolytic Anaemias

Ab against drug eg. Penicillin
Drug attaches to RBC > destroyed
Membrane modification eg. Cephalosporin
Immune complex formation eg, streptomycin > complex attaches to RBC, activates complement > hemolysis.
Auto-antibody production eg. Methyldopa

Transfusion and AIHA
Patient clinical data vital; especially if DAT is positive.
Only transfuse if life-threatening. Ab also lyses transfused RBC.
Packed cells or exchange transfusion to prevent circulatory overload.

#7. THE COMPATIBILITY TEST

Donor RBC vs Recipient's Serum
Tests: patient ABO
patient Rh
patient's antibody screen
donor ABO done again (no matter how many times previously donor has donated)
donor Rh done again (")
patient auto-antibody control (patient serum vs patient cells)
compatibility test (crossmatch).

Request form: write products on form. Indicate whether compatible or not.
Any errors; clerical or Serological can be fatal!
Major crossmatch
Donor cells vs patient serum; to determine ABO, Rh antibodies in patient serum.
Whatever antigens pt. does not posses on their RBC, the antibodies to will be in their serum.
http://en.wikipedia.org/wiki/ABO_blood_group_system

Minor crossmatch (reverse match)
Donor serum vs patient cells.
Seldom done.

Compatibility Test Procedure
Donor cell suspension + patient serum.
Spin immediately; red macroscopically.
If negative: end of crossmatch.
Also patient saline AHG screen (Coombs).
If negative, procedure complete.
If screen is positive, identify ab using panel or quicker screen (all ag's in 2 bottles).
Remove incompatible units, if any.
NB: crossmatch and AHG must also be done @ 37 deg.C. (body temp.) if any ab present.
Also do auto-control @ 37 C. (pt cells vs pt serum).

Common problems
Cold auto antibody
Auto anti-H, auto anti-I.
Pt has both ag and ab; only reacts below 37 C.
Anti-I will only be neg. with ii (cord cells) in panel.
Anti-H only neg. with Bombay cells in panel.
If negative at 37 though; safe to issue.
Note the ID of the antibody.
Warm auto-antibody
Auto anti-e.
Incompatible crossmatch @ 37 deg.C.
Auto control +
DAT +
Avoid transfusion if possible.
T-ag activation
Bacterial infection affecting membrane; exposing RBC T-ag .
Everybody has anti-T.
If poly-agglutination – test for T-ag. on pt cells.

Rouleaux
RBC's stacked like coins
Abnormal globulins, eg. Myeloma
Add saline – rouleaux goes away; not agglutinations.
Whartons Jelly in Cord cells
Causes false agglutination
Irregular antibodies
Allo-antibodies (other than auto-) in patient.
Biggest problem.
Select negative units.
Ab to high frequency Ag
eg. Anti-k (k-ag frequency 99%)
k negative donors make it to the Rare donor file.

Emergencies
? Group not known (X-match not complete)
if known, issue same group.
If unknown: male get O Rh pos.
post-meno female get O Rh pos.
female of child bearing age get O Rh neg.
Never necessary to give more than 1 unit; draw blood specimen > lab for further X-match.

Exchange Tx
Same as infant – NB if mom compatible ABO; not Rh
eg. A pos. baby with anti-D-HDN from A-neg. Mom:
A negative blood used. (usually routinely O negative given)
B pos. baby with anti-D-HDN from A negative mom:
Give O negative blood.

Titres in O donors
Titres of ABO ab's determined in donor sera.
Hi titre – if at 1:64 dilution still positive to test cells, vs Low titre.
Risk of minor incompatibility.
Packed cells – no problem; too little serum.
If whole blood is used, issue low titre; even to O patients.

Autologous Tx
Donate for own use.
Religion
Rare group
Many antibodies (eg. Patients who had many transfusions)
4 units may be collected in 28 days; not very practical.

#8. HAZARDS OF BLOOD Tx

REACTIONS

Types of Reactions

- **Haemolytic**

Intravascular – caused by ABO incompatibility.
Extravascular – hemolysis outside vascular system – inside spleen

Major incompatibility: pt ab to donor cells – most severe
Minor incompatibility: donor ab

ABO most dangerous: always ab in patient.
If ab is not hemolytic, eg Rh > mostly extravascular hemolysis.
Hb > urine
Most severe hemolysis: haemosiderin in urine (black), high bilirubin.
ABO reaction starts after only few ml.

Symptoms

Burning veins, flushed face, fever, headache, lower back pain, chest pain, renal failure, At times Cpt released → coagulation → fibrin clots
DIC
Renal failure
Vasoconstriction
If extravascular; no symptoms evident.

- **Delayed Haemolytic**

Eg. Rh
Kidd
Primary to secondary response takes few days.
Both major and minor incompatibility (resolved with packed cells).

Symptoms

chills, fever
sudden onset of chest pain

Blood samples:

DAT positive (ab already bound to cells in patient)
Haemoglobinaemia (free Hb in serum)

- **Pyrogenic Reactions**

Fever producing
Causes: dried blood; proteins
organisms
endotoxins (use 2x osmosis H_2O)

Symptoms

Severe chills; high temp.
Nausea and vomiting
Severe headaches
Muscle pain
Usually 30-60 min after infusion
Temp. usually returns to normal in 4 hrs.

- **Contaminated Blood**

Bacteria eg. Pseudomonas, Coliforms, Achromobacters
grow at 4 deg.C.; produce endotoxins.
Due to: Poor cleaning of patient's arm
Non-sterile equipment

Open systems eg. Pin holes.

Symptoms
Chills, fever
Aches and pains
Hypotension
Shock
Red skin
May be fatal if not treated
Administer tetracycline
Solution: good technique, check plasma

- **Allergic**

Allergens in donor blood
symptoms: itchy skin, hives
Rash
Edema
Wheezing
Stop transfusion
Give antihistamine (same needle)
Continue transfusion if patient recovered.

- **Air Embolism**

> cardiac arrest
not with plastic bags.

- **WBC Reactions**

Ab in patient, eg patients with Tx history
Not life threatening
Symptoms Phase 1: Flush
Palpitations
Sweating
^pulse rate
Tight chest
Coughing
Phase 2: minimal signs
Phase 3: ^diastolic
Headache
^temp.

- **Citrate toxicity**

Rapid infusion / large quantities
Citrate usually cleared by liver
NB impaired liver function
Decreased body temp.
Babies at risk.

- **Circulatory overload**

Whole blood
Symptoms: Dry cough
Tight chest
Fluid in lungs
Give packed cells.

- **Shock**

Cardiogenic Shock
Heart muscle contracts ineffectively
Anaphylactic Shock
eg. patient with anti-IgA gets IgA antibodies with transfusion.

Symptoms: Laryngial edema
Chills, fever, sweating
Fall in BP.

Septic Shock

contamination

Haemorrhagic Shock

After blood loss:
IV fluid cannot carry O2
Issue RBC and volume expanders
Do not warm blood if high blood loss (500 ml / 5-10 min)
Infuse fluid while waiting for x match.

Vasovagal Shock

1st time donor
mere sight of blood > sweating, low pulse, vomit, voiding
Head between knees

Investigation of Reaction (Role of Tech in management of TRx)

Protocol:
Doctor to report to BTS
Register maintained (serial numbers)

- Clerical check

Patient: pre-Tx sample
post-Tx sample
other samples
Request form
Reaction report
All transfused donor units.

- Visual Check

Hb and Bilirubin in samples

- Urine

Blood, protein, Hb, Bilirubin

- Test on pt. Pre- and post-sample

Bilirubin (pt. May have had high bili anyway)
Repeat blood group
Repeat x match
Do antibody screen
DAT

- Test on donor

Gram stain / culture on pack
Gram stain culture on segment (pack segments for running tests without compromising closed system)
Repeat blood group

Draw up report:
identify reason for reaction
attach to worksheet
Keep as permanent record

TRANSMISSION OF DISEASE

Any pathogen transmitted from donor to patient

Risk lessened by 4 deg.C. storage; immune system; testing of all donor units.
? patient or donor disease

- Syphilis

T. pallidum
Does not survive 72 hr @ 2-10 deg.C. in citrate.
Thus, danger with fresh whole blood
All units get VDRL test.

- Malaria

Plasmodia survive cold storage many weeks; even to – 70 deg.C. several months.
Sometimes cannot detect parasite.
2 approaches:
endemic regions: Travel > no donation for 6 months
if donor has had malaria > 3 years symptom free.

- Hepatitis

Most dangerous from blood transfusion is Hep. B
History of hepatitis > exclude donor
Exclude high risk: prisoners, drug addicts, multiple transfusions, low income (socio-economic)
ELISA done on every donation.

- HIV / AIDS

Characterized by severe opportunistic infections and / or tumors eg Kaposi
T4 helper depleted > severely impaired cell-mediated immunity.
Not allowed to differentiate homo- / heterosexual in S.A.
Exposure → diagnosis = 29 months
Factor VIII higher risk (pooled from multiple donors)
NB paid vs unpaid donors. Voluntary = lower risk.
Test on every donation. High sensitivity. Some false positives do occur (ie low specificity)
P24 ag routine. Very sensitive for virus. Can get false pos.

Summary:
Screen donors; test every donation; repeat tests.

#9. MEDICO-LEGAL; GENERAL ASPECTS

Blood Transfusion services may be involved in litigation due to mistakes by staff.
Subject to criminal proceedings if negligence involved.
Rules must be laid down.
Employee involved has access to legal advice.

Lab Precautions

No smoking, eating
gloves
no mouth pipettes
care with needles
lab coats
treat all samples as potential hazard
cover up open cuts
wash hands
remove lab coat before leaving lab.

Product Recall

Due to complaint of a product
written instruction required
contact all organizations involved.
Record batch nr, serial nr etc.
Establish fate; keep in quarantine
Blood product: inform Inspector of Anatomy.
Fractionated product: inform Medicines Control Council
State clearly the responsibility of: staff, supervisor, Responsible officer, Organization Head, Legal advise; ie Job descriptions

Other roles of Blood Lab: Investigation of disputed paternity using blood typing. Older method. Now DNA polymorphisms used.

: Forensics.

Forensics

Like paternity; cannot include; only exclude.
Steps: Is it blood ?
Is it human ?
Determine blood groups
May use saliva if secretors.
Anthropology studies – changing frequencies.
Polymerase Chain Reaction (PCR) used to amplify a segment of DNA million fold; eg. Nail scraping, hair, semen. Theoretically any sample with one cell.
KIT: DQA-PCR (HLA-Class II)

#9. QUALITY CONTROL

Clerical errors most NB in reactions.
Always double check clerical info.
Always write “ Neg “ on crossmatch form not “-”.
Write up immediately, not from memory.
Equipment:
Method for use readily available
Calibrated, maintained regularly.
Reagents:
Test each reagent every day with pos. and neg. control.
SOP's for all reagents available; read and signed.
Personnel:
Job descriptions: qualification; registration, responsibility, duty, accountability.
Evaluation system: proficiency testing (internal and external)
Training programmes
Components:
SOP for all preparations
QC of all products
Representative sampling
Also:
pleasant working conditions
Do not interrupt other workers: They have somebody's life in their hands.