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Blood Transfusion

- AUTOLOGOUS BLOOD TRANSFUSION
- ALLOGENIC BLOOD TRANSFUSION

Transfusion Algorithm

- Avoid Transfusion : medical and surgical
- Alternatives

  replacement fluids: crystalloids and non plasma colloids over plasma

  pharmacologic agents to reduce bleeding
- Autologous donation
- Minimize exposure to allogeneic transfusion

Transfusion Algorithm

Is it possible to avoid transfusion ?

Medical:

Treat underlying cause of asymptomatic anemias:

Nutritional deficiencies-supplements

Chronic GI bleeds-medications

Renal failure- erythropoietin

Transfusion Algorithm

Is it possible to avoid transfusion?

Surgical:

Excellent surgical skill (avoid tissue trauma, attention to hemostasis, utilize avascular plane etc)

Use of topical hemostatic agents in OT

such as Fibrin Glue

Transfusion Algorithm

- When transfusion is deemed necessary, a physician must obtain informed consent from patient.

Transfusion Algorithm

Strategies to minimize exposure to allogeneic transfusion

1. **replacement fluids- crystalloids and non plasma colloids**
2. pharmacologic agents to reduce bleeding
3. Autologous Transfusion

Acute Blood Loss

Mild 10-15%	Contraction of great veins	None or mild and transient
Mod 30%	Decreased cardiac output tachycardia	Thirst hypotension weakness etc
severe>30%	All of the above plus < 50% card op	Air hunger, Loss consci lacticacidosis

Acute Blood Loss

Estimate % Blood Loss	Treatment Vox San 1992;63:241
Up to 20%	Volume replacement (crystalloid)
20-50%	<3RCC+ volume rep
50-90%	>3RCC+colloid+?FFP
>90%	RCC+FFP+PC(if>1.5 body volume)

Replacement Fluids

- Crystalloids - Saline, DNS, Ringer's lactate not maintain blood vol longer
- Colloids - Hydroxyethyl starch: Pentaspan and Hexpan, Dextrans and Gelatins - maintain blood volume longer

Pharmacologic Agents

- Recombinant Growth Factors
  1. Erythropoietin EPO
  2. Colony Stimulating Factors CSF
- Hemostatic vasopressin agents – releases FVIII & vWF from endothelium
- Antifibrinolytic agents:
  1. Epsilon aminocaproic acid EACA etc.
- Recombinant VIIa

AutologousTransfusion

- Preoperative Autologous Donation PAD
- Intraoperative hemodilution
- Intraoperative salvage
- Postoperative salvage

AutologousTransfusion

- Although not completely risk free but safe
- Autologous Blood donation should be the transfusion therapy of choice,
- Or at least, fully and carefully considered.

Who is qualified to be an Autologous Blood donor?

- Not anemic (Hemoglobin = 11 grams, slightly lower than required of a regular Blood donor, i.e., 12 grams).
- No medical condition that could cause problems during or after the Blood donation process.
- Children weighing over 30 kg
- having planned surgery that routinely requires a Blood transfusion (except in cases where long term storage is desired).
- Those who have veins large enough for the procedure.

Advantages of Autologous RBC

- Prevents transfusion associated diseases
- Prevents alloimmunization
- Reduce demand on donor units
- Reduce some risk of transfusion reaction eg. Febrile, allergic and hemolytic Tx Rx
- Psychological benefits to some patients

Disadvantages of autologous RBC

- Similar risk of bacterial contamination
- Similar risk of clerical error
- More costly
- More wastage
- Anxiety to some patients
- Higher incidence of adverse reactions in donation
- Perioperative anemia and side effects of iron supplementation

Intraoperative Blood Collection (Salvage System)

- Salvage of shed blood from sterile surgical field, washed with saline to remove debris and anticoagulant, concentrate (Hct 50-60)and re-infuse using a microscreen filter (40 microns)
- Surgical procedures using large quantities of RBC eg. open Heart, liver transplant and vascular surgeries are most cost effective
- Complications are rare but have been reported- DIC, hemolysis due to high pressure suction and mechanical compression in roller pumps

Postoperative blood collection

- Recovery blood from surgical drains followed by re-infusion with or without processing (limit to 1400ml)
- Most common in orthopedic procedures such as hip or knee replacement.

Blood Substitutes (Artificial blood)

Ideal: good O2 carrier, non immunogenic, non toxic, storage stable, acceptable in vivo retention(half life in weeks or months), non infectious, low viscosity for reperfusion of ischemic organs during strokes, MI and in organ transplants, can be massively produced to reduce cost.

NO SUCH LUCK SO FAR!

Blood Substitutes

O2 Carrier	Trade Name, Manufacturer
• Perfluorocarbons	• Fluosol-DA, Green Cross
• Recombinant HB	• Optro, Eli Lilly
• Polymerized HB	• Hemopure, Biopure
	• PolyHeme, Northfield Lab

Donor Selection & Blood Collection

Blood Donation Process

- Donor area should be
  - attractive
  - accessible
  - well lighted
  - comfortable ventilated
  - clean
- Phlebotomist should be friendly, understanding, professional & well trained

Registration

The following information should be included

1. Date and time of donation.
2. Name: Last, first (and middle initial if available).
3. Address: Residence and/or business.
4. Telephone: Residence and/or business.
5. Gender.
6. Age and/or date of birth. Blood donors must be between 18 – 60 years of age
7. No age limits for autologous blood collection, each patient must be evaluated to determine if collecting blood will be safe.
8. A record of reasons for previous deferrals
9. A deferral registry should be maintained in the blood bank

Information Provided to the Prospective Donor

- All donors must be given educational materials informing them of the clinical signs and symptoms associated with HIV infection and AIDS, of high-risk activities for HIV transmission, and of the importance of refraining from donating blood if they have engaged in these activities or experienced these signs or symptoms.

- Consent must be taken and documented that donor has read the material and understand each & every points
- Following information must be provided
  - unexplained wt loss
  - night sweat
  - blue or purple spot – kaposis sarcoma
  - swollen LN
  - temp > 100º F for more than 10 days
  - persistent cough & shortness of breath
  - persistent diarrhea

- Donor must be told that his/her blood will be tested for HIV, HBV, HCV, syphilis, malaria
- He/she must be asked that if his/her test result found abnormal should be intimated on phone or by post confidentially

Donor selection

- Donor selection is based on medical history & physical examination
- History & examination should be carried out in a manner that proper privacy must be maintained

BASIC REQUIREMENTS

- Be in generally good health and feeling well.
- Be at least 18 years of age; upper age 60
- Weigh at least 45 kg.
- Pulse: 80 to 100 beats/min and regular.
- Temperature: Should not exceed 99.5 (37.5c).
- Blood Pressure: acceptable range is 160/90 to 110/60.
- Skin: the venipuncture site should be free of any lesion or scar of needle pricks indicative of addiction to narcotics or frequent Blood donation (as in the case of professional Blood donors).

DO NOT DONATE BLOOD IF:

- You have ever tested positive for HIV,
- You are IV drug user
- You are a man and have had sex with another man, even once,
- You have hemophilia or another Blood clotting disorder and received clotting factor concentrate,
- You have engaged in sex for drugs or money since 1977,
- You have lived in western Europe since 1980, (CJD)
- You have been in including jails, prisons and/or detention centers for more than 72 hours in the last 12 months,

MEDICAL CONDITIONS

- Accident & Injury: can donate if otherwise healthy
- AIDS: can not donate
- Babesiosis: can not donate
- Blood disorders or bleeding tendencies: can not donate
- Blood Pressure: acceptable range is 160/90 to 110/60.

- CJD even family history can not donate
- Viral infections: defer donation until four weeks after recovery
- Colds, fever, flu, sore throat: can not donate until symptoms (sore throat, cough, respiratory infection, headache) are completely gone
- Diabetes: can donate if treatment is by diet control and condition is stable; defer donation if on medication
- Filariasis: can not donate

- Gonorrhea/Syphilis & other STD: defer donation for one year after complete recovery
- Heart attack: can donate if greater than one year since, and no symptoms present, - carefully evaluated
- Heart surgery, bypass surgery or angioplasty: can donate one year after surgery, if no history of heart attack, and the donor is on no medication for the heart (aspirin is okay)

- Hepatitis: Hepatitis or undiagnosed jaundice after age ten; can not donate.
- Leprosy: can not donate
- Malaria; had Malaria in last three years: defer donation for three years after full recovery
- Pregnancy and Miscarriage: can donate after six weeks of full term normal delivery.
- First or second trimester miscarriage can donate if stable
- Sickle Cell Trait: can not donate

- Seizures in the last five Years: can not donate
- Surgery (all): can donate after healed and released from physician care.
- Tuberculosis: can not donate until two years after complete cure

MEDICATION GUIDELINES

- Acetaminophen may be taken in normal moderate doses before any Blood donation
- Allergy medication: can donate
- Antibiotics: 72-hour deferral after infection is healed
- Anti-inflammatory drugs (Ibuprofen): may not be taken within 24 hours before a platelet donation
- Aspirin taken within 36 hours platelet concentrate should not be prepared.

- Birth control pills: can donate
- Blood pressure medication & antidepression medication: can donate
- Diabetic medication - can not donate
- Diuretics: can donate
- Female hormone pills: can donate

IMMUNIZATION EXCLUSIONS

- Polio, mumps, smallpox: two-week or more deferral
- Rubella or Rubeola (types of measles): four week deferral
- Tetanus, diphtheria, flu, Hepatitis B: can not donate until any reaction is over

OTHER POSSIBLE RESTRICTIONS

- Acupuncture: one-year deferral
- Alcohol: defer donation if consumed in last 12 hours
- Body piercing: one-year deferral
- Dental work - Cleaning and fillings: one-day deferral; Root canal: three-day deferral after work is complete
- Ear piercing: can donate if the piercing was performed in a doctor's office (with written verification) otherwise, one-year deferral

- Electrolysis: defer donation for one year
- Hepatitis exposure: one-year deferral
- Menstruation: can donate
- Rape: one-year deferral
- Smoker: can donate
- Tattoo in the last 12 months: one-year deferral
- Transfusion: defer donation by one year if undergone transfusion with Blood products. Can donate if undergone autologous transfusion only

Physical examination

- **GC**
- **Weight > 45 kg – 350 ml**
  > 60 kg – 450 ml
- **Temp –**
- **Pulse 50 – 100 /min**
- **BP – acceptable range is 160/90 to 110/60.**

- **Hb – 12.5 g%**

Blood collection

- Donor should be asked about meal with in last 4 hrs.
- Clean Venepuncture – little trauma,
- Blood should be collected within 8 min

Care for donor after collection

- Apply pressure on phlebotomy site
- remain reclining on donor couch for few min. under observation
- Allow donor to sit under observation
- Refreshment at least juice should be given
- Ask to drink more fluid in next 4 hrs
- Avoid consuming alcohol untill eat something
- Don’t smoke for 30 min
- If bleeding raised arm and apply pressure
- If fainting or dizziness lie down or sit with head between knees
- If any symptom persist inform or come to BB
- Resume all normal activity if OK

Adverse donor reaction

- Most of donor tolerate well
- phlebotomist must be trained for CPR
- Syncope (vasovagal attack) after seeing blood – sweeting, dizziness, loss of consciouness, fainting -cold calmy skin BP falls PR slow
- Care – remove tourniquet stop bleeding. Put ice pack on head & back of chest, loss tight clothing, monitor BP & pulse
- If prolong infuse NS
- If nausea vomiting – make donor comfortable, breathe slowly, turn head to one side
- Other such as convulsion etc rare and manage accordingly
- If cardiac arrest CPR call emergency immediately

How blood components are prepared

- Use of triple bags. One primary, one empty & one SAGM bag (350 or 450 ml)
- Blood collected in primary bag
- Left for 2 hrs at RT if PC has to make or at 4º c it cryo is to make
- If PC – low spin – 1850 rpm x 9 min at 22 º c – 2 layers PRBC & PRP
- Separate by plasm expressor plasma in empty bag, add SAGM in PRBC addition of SAGM – shelf life increases from 35 to 42 days – detach PRBC & store at 4º c

How blood components are prepared (contd.)

- Second spin hard – 3850 rpm x 7 min at 22º c
- Platelet settled at bottoms
- Express plasma in second bags platelets remain in bags
- Store PC at 22º c with continuous agitation
- Store plasma as FFP freeze at – 30º C or more for 1 yrs
- After 1 yrs labeled it as CPP at stored for 7 yrs

How blood components are prepared (contd.)

- For cryo after collection keep at 4º c for 2 hrs
- Hard spin 3850 rpm x 7 min at 4º c
- Two layer cryo rich plasma and PRBC
- Express plasma in empty bag push SAGM in PRBC stored at 4º c for 42 days
- Deep freez CRP at – 80º c for 72 hrs
- Thaw at 4º c for 24 hrs recentrifuge hard spin curdy ppt of cryo at bottom express out cryo poor plasma and cryoppt remain in bag stored deep freez at -80º c for 1 yr

Pre transfusion testing & Cross Matching

Purpose – to select component that will not cause harm to the recipient and have a maximum survival when transfused.

Protocol for Pre transfusion testing

According to AABB Standard following procedure should be opted.

- Positive identification of recipient & recipient blood sample
- ABO group and Rh typing of recipient blood
- Red cell Ab detection for clinically significant Ab using recipient serum/plasma
- Comparison with previous record, if any

AABB Standard (Contd.)

- Confirmation of ABO by reverse grouping
- Confirmation in case of Rh –ve case
- Selection of component appropriate for recipient
- Performance of serological cross matching
- Labeling with recipient’s identification

Transfusion Request

- Request should be properly filled with all relevant information for recipient
- Component needed, quantity & any other request such as irradiation etc.
- Responsible doctor's signature with name and identification code.
- Telephonic request should only in urgent cases but should be documented.
- Most of the HTR – due to clerical error

Blood Samples

- Two tubes, one plain (5 – 7 ml) other EDTA (2 ml).
- Properly labeled with recipient full name and hospital number ward/bed.
- It should be identified & checked by blood bank staff
- If any discrepancy – request should be return but not the sample & fresh sample requested. (Sample once entered BB should not go out).

Blood Samples (Contd.)

- After finishing the test or if test required after some time it should be preserved at 4ºC.
- Sample should not be 3 days old.
- Recipient’s sample and donor red cell kept for at least 7 days after transfusion.

Serological Testing

- ABO & Rh typing
- Testing for expected & unexpected Ab
- BB incharge discretion to decide Ab panel

Cross Matching

- Unless it is urgent, cross matching should be performed
- If uncross matched blood required it should be noted on the request and should be signed by consultant, should be approved by BB incharge.

Cross matching (Contd.)

- What is cross matching – it is procedure to demonstrate ABO compatibility & compatibility among clinically significant Abs.
- if on Ab screening, any significant Ab detected, cross matching should include antiglobulin test, other wise a simple spin cross match can be performed to reduce TAT & workload and reduce reagent cost.

Cross Matching (Contd.)

- Repeat testing of blood group on donor's sample
- Checking of label on the bags
- Cross matching should be performed from the segment of the bag not from the sample stored separately any discrepancy should be resolved before.

Suggested procedure for routine cross matching.

- Segment from the bag is taken
- Cell to be washed & cell suspension of 2 – 5% with NS.
- Simplest technique - simple spin technique - serum mixed with cell suspension at room temp. & centrifuged immediately.

Suggested procedure for routine cross matching (Contd.)

- This is designate to detect ABO incompatibility between donor and recipient.
- When pt. have no previous or clinically significant antibody this is sufficient.
- This detect anti M, N & P also as done at RT

Method (Immediate spin technique)

- Pt. serum or plasma not more than 3 days old.
- Reagent – normal saline, donor cell sus. 2 - 5%.
- Add cell susp. to pt. serum in a tt, centrifuged and examine for agglutination.
- Interpretation – gross or microscopically seen agglutination – incompatibility (not matched)

At 37ºC

- If IS shows no agglutination incubate the tube at 37ºC for 30 min
- See for agglutination
- If gross or microscopic agglutination seen (incompatible)
- If no agglutination go for AHG phase

AHG Phase of Cross Matching

- Other alternative is to do IAT (ICT) to detect Ab to red cell Ag.
- Here first IAT is done with pt serum and donor's 5% cell suspension instead of known O red cells.
- Here antigenic characteristic of red cell and Abs status of plasma - not known
- Demonstrate if any sort of Ag-Ab interaction is there.

Method

- Prepare 5% of washed donor's red cell susp.
- Take 2 drops of pt serum in a tt
- Add 1 drop of donor's cell susp. Mix centrifuge and read, record result
- Add 2 drop of bovine Alb incubate at 37ºcx30 min
- Read for hemolysis and agglutination & record result.
- Wash 3-4 times with saline decant and add 2 drops of AHG sera and mix

Method (Contd.)

- Centrifuge mix and examine for agglutination record result
- If all results are negative add 1 drop of CCC centrifuge and examine if still result is negative repeat whole procedure otherwise agglutination will occur after addition of CCC.
- No agglitination in any phase upto addition of AHG but agglutination is seen after addition of CCC this be reported as compatible blood

Human Blood Group

- Land Steiner in 1901 discovered the blood group.
- After that a vast discovery in this field attributed
- At least 100 Ag defined
- 15 well defined system of Ag
- Eg- in order of discovery - ABO, MNS, P, Rh, Lutheran, Kell, Lewis, Duffy, Kidd, Diego, Yt, Li, Xg, Dombrock, and Colton.
- Some are specific for certain racial group – Diego in South African Indian, Japanese and Chinese.
- ABO and Rh are most commonly have clinical importance.
- Others are less important because Ag are week and corresponding Abs are not present & occur only after usually multiple transfusion.

Human blood groups are named after the presence of Antigens on Red Cell surface and their corresponding Antibodies in sera.

There are 2 type of Abs

1. Naturally occurring Abs
2. Immune Abs

Naturally occurring Abs

- These Ab normally present in human sera without exposure to corresponding Ag.
- Eg. Anti A, Anti B
- Other - Anti A^1, Anti H, Anti P^1, Anti Lewis, and Anti I.
- Anti M & Anti N in some part of Africa.
- Anti i in new guinea & south America.
- Naturally occurring Abs – High Mol. Wt. (900,000 Dalton) IgM type.
- React with appropriate red cell Ag at lower temp rather at 37ºC. (Cold in nature)
- It will react in saline media.

How these Abs are produced?

- Person contains A Ag have B Ab
- Hypothesis – configuration that confer A & B Ag determines on red cell surface also exist on other biological entities – such as bacterial wall – exposed to these bacteria – constant exposure to A or B like antigen structure – Ag which are not present considered as foreign and Abs formed against them.
- Thus A antigen – B Ab formed, B Ag – A Ab formed for O group no Ag both Abs formed, for Abs group both Ags present no Abs formed.

Immune Antibodies

- Produced by the introduction of red cell allo-antigen not possessed by the individual.
- Introduced by parenteral transfusion, IM or Intradermal injections and passage of fetal cell in mother circulation during pregnancy.
- Most Rh Abs (except some for Anti E), most Kell, Duffy and Kidd are immune in origin.
- First they are IgM in nature later they change to IgG types. IgG smaller 140,000 Dalton
- Warm in nature, combine with appropriate Ag rapidly at 37ºC.

ABO Blood Group System

- Four main group AB, A, B & O
- This based on the presence of 2 Ag (A & B) on the cell surface.
- Ag – under control of A and B genes which with third allelomorhic gene O – inherited as simple dominants (O gene exert no effect on basic antigenic structure). ABO locus on Ch 9
- A & B substance on the red cell are glycosphingolipids.
- Basic carbohydrate substance is called H Ag & its formation is controlled by another gene which is different from A & B gene.

ABO Blood group System (Contd.)

- H gene on Ch 19
- H Ag present virtually on all red cells and their presence is influenced by A & B gene.
- The presence of A & B gene result in addition of sugar to the H antigen to produced A or B antigens.
- The incidence of ABO phenotype varies in different population
- Common blood group in our population is B and less is AB

ABO Blood group System (Contd.)

- Two subgroup of blood group A- A^1 & A^2
- 20% of blood group A & AB are A^2 & A^2B rest are A^1& A^1B.
- A^2 gene seems to convert less H Ag to A than does A^1 gene.
- When human red cell exposed to anti H they react in the following order of strength $O>A^2> A^2B>B>A^1>A^1B$
- Label of blood groups are according to the presence of Ag on the RBC.

Bombay Blood Group

- Very rarely an individual red cell are not agglutinated by anti A, anti B or anti H (Bombay blood group) .
- Bombay red cell consequence of homozygosity of gene h, allelomorphic to H.
- These individuals can not formed basic H antigen and thus A and B antigens can not be expressed even A or B genes are present normally.
- Anti A, Anti B and anti H all active at 37ºC are present in their sera.
- Symbol O_h for this blood group.
- This blood group person on testing blood group apparently shown O blood group because they will not show any agglutination with A or B antisera but show incompatibility with O group blood.
- Bombay blood group will be compatible with only Bombay blood group donor.

Rh System

- Original Ab was raised by injecting red cells of **RHESUS** monkey into rabbit or guinea pig and testing the resulting sera against human red cells.
- Clinical Importance – Rh negative individuals are relatively easily stimulated to form Rh antibodies if transfused with Rh positive blood or in the case of pregnant lady if fetus is Rh positive and there is any leak through placenta into maternal circulation.

Rh System (Contd)

- Sufficient for clinical importance to decide that Pt. is Rh +ve or Rh –ve.
- This can be decided by testing RBC with commonest type of Rh Ab – anti D
- Commonest type of antigens are D or d C or c, and E or e.
- Persons having gene cde/cde are called Rh negative.
- Less common Ag are C_w, D_u and e_s.

Testing for Blood groups

- Material required – EDTA blood, Serum/plasma, anti sera, known blood cell suspension.
- Make the cell suspension of 5% by washing the cell by saline 4 – 5 times.
- Antisera – anti A, anti B and anti D commercially prepared anti sera are available in market. Anti AB also available but of less used
- Previously a color code was used

  anti A - blue

  anti B - yellow

  anti AB - orange

  anti D - colorless
- But now it is of less importance

Testing for Blood groups (Contd.)

- **TUBE METHOD** – take 3 test tubes (90 x 10 mm) and label A, B & D.
- Add anti sera A, B & D in respective tt
- Add 5% cell suspension in all the 3 tt & mix well.
- Leave for some time and examine for agglutination – grossly as well microscopically.
- Interpretation –

Test tube A shows agglutination – blood group A

Test tube B shows agglutination – blood group B

Test tubes A &B show agglutination – blood group AB

Test tubes A & B show no agglutination – blood group O

Test tube D shows agglutination – blood group Rh +ve

Test tube D shows no agglutination – blood group Rh -ve

Testing for Blood groups (Contd.)

- SLIDE METHOD – Take 3 slides label them with A, B & D
- Put 1 drop of anti sera A, B & D on their respective slides
- Put one drop of cell suspension on each slide mix well and see for agglutination grossly as well as microscopically.
- Interpretation-

Slide A shows agglutination – blood group A

Slide B shows agglutination – blood group B

Slides A &B show agglutination – blood group AB

Slides A & B show no agglutination – blood group O

Slide D shows agglutination – blood group Rh +ve

Slide D shows no agglutination – blood group Rh -ve

Reverse grouping

- Make cell susp. (2 – 5%) of known A & B cell
- Take Pt. Sera in 2 TT marked A & B
- Add cell susp. A in A TT and B in B TT
- Mix & centrifuge
- Check for gross & microscopic agglutination

Interpretations

- Agg in TT A – B group
- Agg in TT B – A group
- Agg in TT A & TT B – O group
- No Agg in TT A & TT B – AB group

Interpretation of ABO group

Blood Group	Antigens	Antibodies
A	A	Anti B
B	B	Anti A
AB	AB	No
O	No	Anti A, Anti B

Week D (D_u) procedure

- No agg in anti D after immediate spin add one drop of Anti D and incubate for 15 min at 37ºC
- Examine for agglutination
- If Test shows agg & control no agg - Rh +ve (No need of further test)
- If still no agg then wash 3 - 4 times with saline decant completely and add 2 drop of AHG mix centrifuge x 30 sec resuspend see for agglutination (gross & microscopically)
- If agg. D_u positive (Rh positive)
- If negative add CCC mix centrifuge and see for agg if negative repeat the procedure

- NOTE:

  When mother is Rh negative and cord blood for new born give positive DAT suspect false negative result with anti D because Rh + new born red cells are covered with maternal anti D leaving no D site available

Interpretation of Rh group

Anti D	Du (AHG)	Rh control	Interpretation
+	N/A	-	Rh Positive
-	+	-	Rh Positive
-	-	-	Rh negative
-	+	+	Invalid test
+	N/A	+	Invalid test

Grading for agglutination Rx

Appearance	Grade
Red supernatant no intact cell	Hemolysis (H)
Single agglutinate (one button)	++++ (4+)
Number of large agg	+++ (3+)
Large agg. With multiple small clump	++ (2+)
Number of small agg cloudy red background	+ (1+)
Easily dispersed very small agg. cloudy red background	Week agg
Microscopically agglutination Grossly negative	Microscopic agg

There are various other tests performed in the blood bank –

- Direct Coomb's Test or Direct Agglutinin test (DCT or DAT)
- Indirect Coomb's Test or Indirect Agglutinin test (ICT or IAT)
- Pre transfusion testing & Cross Matching
- Antibody titration
- Antibody screening
- Antibody detection

Anti Human Globulin (AHG Sera)

- AHG sera – injecting human globulin to animal produces antibodies to foreign protein
- After this, animal serum adsorb to remove unwanted agglutinin
- It will react specifically with human globulin and called AHG sera
- AHG sera – anti IgG antibodies to several compliment components

Coomb's check cell (CCC)

- This is IgG sensitized red cell, should react with AHG sera indicating that AHG sera is functioning
- it indicates that AHG is really added and it is not neutralized

Direct Agglutinin Test (DAT)

- Used to demonstrate in vivo coating of red cells with antibody or compliment – IgG or C3d
- IgG Ab – incomplete Ab – AHG Serum react with IgG and then agglutinate red cells
- Washed red cells are directly tested with AHG.
- 3 - 5% cell suspension is added to AHG sera centrifuge and read for agglutination

DAT (Contd.)

- Used to demonstrate Ab coated on the surface of RBC due to in vivo sensitisation.
- Used to investigate AIHA, drug induced hemolysis, HDN & alloimmune reaction to recently transfused red cell

DAT procedure

- Material : normal saline, Polyspecific AHG, monospefic (IgG, C3d), CCC, Coombs control cell.
- Procedure:

Add 2 drop of cell suspension with three tt marked with AHG, IgG, & C3d, washed

- Poly specific DAT – add AHG to dry cell button, mix centrifuge if negative incubate for 10 min if still negative add CCC if still negative repeat test. No agg before CCC - DAT negative
- If AHG +ve perform mono specific DAT

- Mono specific DAT – add 2 drop of anti - IgG and anti - C3d in specific tube with cell button, mix centrifuge see for agglutination
- If DAT +ve with all, *perform 6% albumin control to rule out presence of spontanous aglutination*
- If anti - C3d negative incubate for 10 min see for agg.
- To any – ve IgG add CCC incubate, see for agg it should be +ve otherwise repeat test
- To any – ve anti - C3d add compliment control cell incubate, see for agg it should be +ve otherwise repeat test

Indirect Agglutinin Test (IAT)

- Used to demonstrate the Ab that are present in the serum (In vitro demonstration)
- Here serum/plasma is incubated with washed 5% suspension of O +ve red cell
- Incubate at 37ºc for 10 min.
- Wash with NS to remove excess Abs

IAT (Contd.)

- Now this RBC are coated with IgG anti Rh Abs of the pt (if Ab are present)
- Add 2 drop coomb's sera (AHG sera) Incubate 37ºc x 10 min centrifuge
- Agglutination occur when AHG is added indicate that antibody is bound to specific antigen present in the serum (+ve result)

IAT Procedure

- Saline IAT
- Add 2 drop of serum/plasma in tt
- Add 1 drop of *saline suspended* cell sus. Of group O+ cell mix centrifuge
- Observed for hemolysis/ agg
- Incubate at 37ºc centrifuge see for agg x 30 – 60 min
- Wash cell 3 – 4 times and completely decant the final wash (dry button)
- Add AHG mix well centrifuge observe the agg if –ve add CCC if still negative repeat test

1. Other, albumin or LISS can be added

- Bovine albumin 22% or 30%
- LISS : add 1.75 g of NaCl + 18 g of glysine in 1l flask
- Add 20 ml phosphate buffer (add 11.3 ml 0.15 M KH_2PO_4 and 8.7 ml of 0.15 M Na_2HPO_4)
- Add DW to 1 l adjust pH 6.7 $\pm$ 0.1 by adding NaOH
- Add 0.5 ml sod. Azide as preservative
- Procedure – add 2 drop of bovine alb. Or LISS after adding plasma/serum before adding saline suspension of O cell

2. Other LISS suspended red cell used

Interpretation

- Agg. Or hemolysis after 37 c incubation - +ve result
- Agg. Or hemolysis after adding AHG - +ve result
- No agg in any time –ve result
- Positive result indicate sensitization with particular types of antibodies present in the serum