Blood processing and some lab investigations related to blood transfusion in pediatric practice

dr

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Goals Of Blood Collection

- Maintain viability and function
- Prevent physical changes
- Minimize bacterial contamination

?In What to collect blood

BULB	FUNCTI ON	USED FOR
EDTA	Chelates Ca preserves cellular elements	CBC,Reticulocytes, ESR,G6PD, Hb electrophoresis
Trisodium citrate	Converts Ca into non-ionized form	PT, APTT, TT Fibrinogen etc
Flouride	Enzyme poison. Inhibits Glycolysis In RBC (Glucose destroyed at 5% / hr)	Glucose

When & How to get Serum & Plasma

- ***Serum sample***
  - **blood collected without any anti-coagulant and centrifuged**
  - **clear supernatant fluid devoid of any fibrin products**
- ***Plain bulb***
  - **Most enzymes,**
  - **Biochemical LFT,KFT,**
  - **S Electro.**
  - **Serological :Widal, VDRL**

- ***Plasma sample***
  - **blood collected & mixed with anticoagulant and centrifuged**
  - **clear supernatant fluid with thrombosis inhibited. Most satisfactory sample. No changes occur in blood**
- ***Heparinized bulb***
  - **PH, NH4, RBC levels, Plasma cortisol, testosterone, globin,cholinesterase**

CRITERIA FOR BLOOD DONATION

- Preferably regular donor
- Weight
  - >55 Kgs
- Good venous access
- Prior investigations required
  - FBC
  - VDRL
  - HbsAg
  - Anti HIV
  - Anti HCV
  - Serum lipid profile

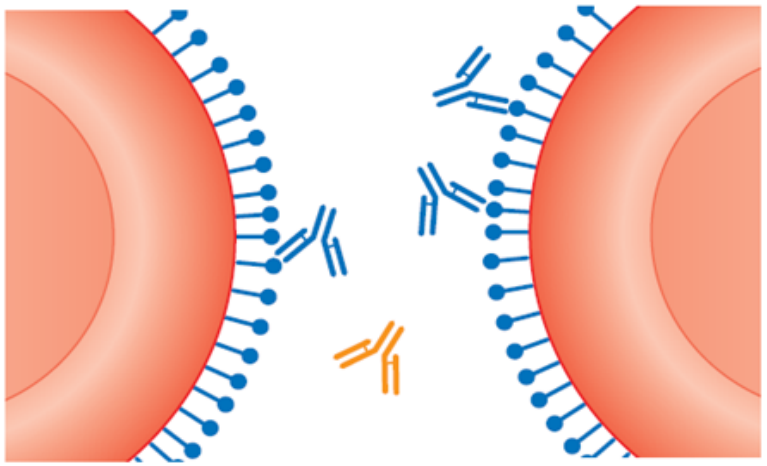
Rejection of Samples

1. **Clotted** - failure to mix or inadequate mixing of samples collected into an additive tube. The red cells clump together making the sample unsuitable for testing.
2. **Insufficient sample (QNS)** - certain additive tubes must be filled completely. Incorrect blood to additive ratio will adversely affect the laboratory test results. When many tests are ordered on the same tube be sure to know the amount of sample needed for each test.
3. **Wrong tube collected** for test ordered. Always refer to procedure manual when uncertain.
4. **Improper storage** - certain tests must be collected and placed in ice, protected from light, or be kept warm after collection.
5. **Improperly labeled**

Rejection of Samples

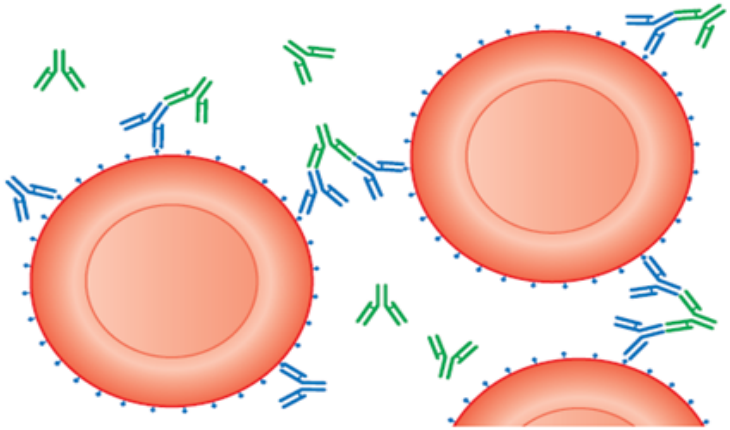
1. **Hemolysis** - this is usually caused by a procedural error such as using too small of a needle, or pulling back to hard on the plunger of a syringe used for collecting the sample. The red cells rupture resulting in hemoglobin being released into the serum/plasma, making the sample unsuitable for many laboratory tests. The serum/plasma will appear red instead of straw colored.

DIRECT ANTIGLOBULIN TEST (DAT)



Cells coated *in vivo*

Washed to remove unbound globulins



Addition of anti-human globulin (AHG) promotes agglutination after centrifugation

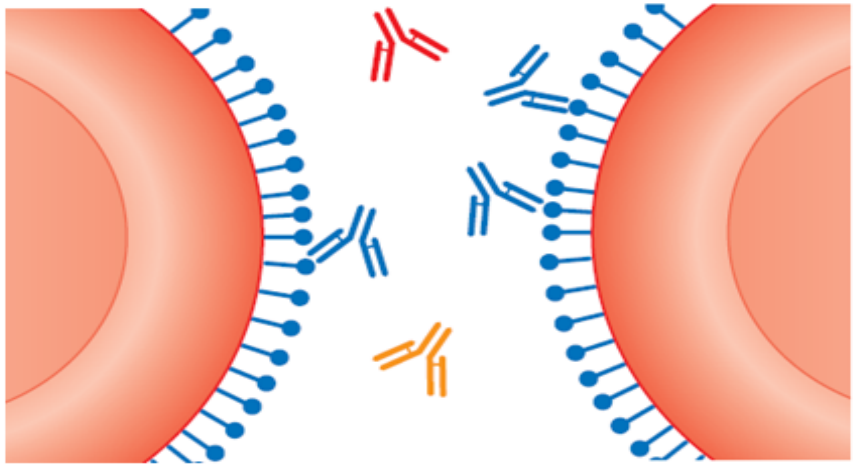
Blood Sample

- Whole Blood Sample - It should be as fresh as possible not more than 24 hours old,
- otherwise, the sample should be taken in EDTA.

Procedure of DAT

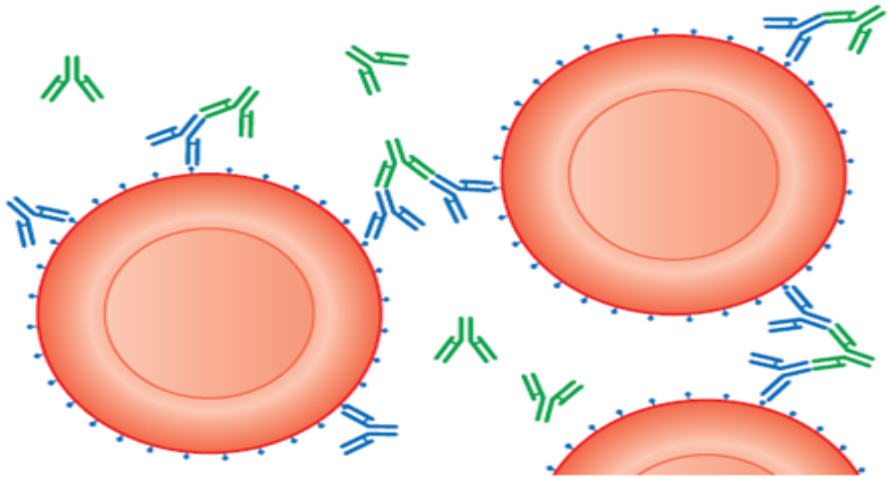
1. Take 2-3 drops of blood to be tested in a clean labeled tube.
2. Wash the red cells 3-4 times in a large volume of saline to remove free globulin molecules. Remove all supernatant after each wash. Completely decant the final supernatant wash.
3. Add 2 drops of polyspecific AHG serum in 1 drop of sensitized washed red cells or in 1 drop of 3-5 % suspension of sensitized cells immediately.
4. Mix, Centrifuge at 1000 rpm for 1 minutes immediately.
5. Gently shake the tube to dislodge the cell button and see for agglutination, use optical aid if needed, Record the result.
6. Add 1 drop of IgG coated red cells to a negative test. Mix, centrifuge at 1000 rpm for 1 min. Immediately look for agglutination. If a negative result (no agglutination) is obtained the test result is invalid and whole test should be repeated. If agglutination is obtained, the result is valid.

Indirect antiglobulin test



Serum with specific antibody mixed with reagent red cells

Washed x3 after incubation to remove unbound globulins



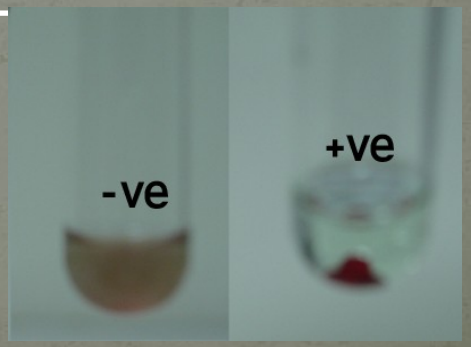
Anti-human globulin (AHG) added to promote agglutination on centrifugation

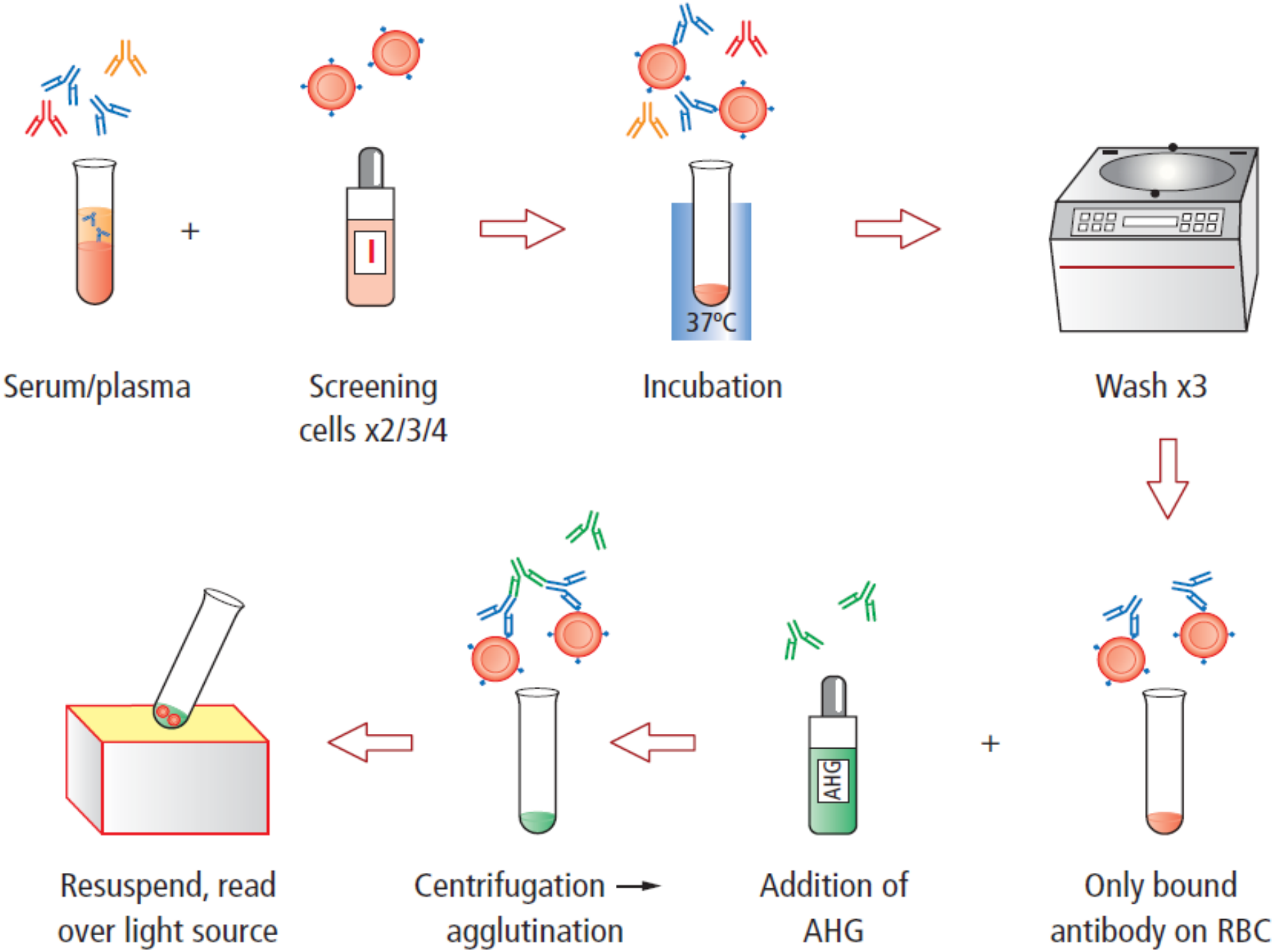
:Procedure

1. Place 2-3 drops of the test serum in a tube. Serum should be fresh for detecting complement components and complement binding antibodies, otherwise, fresh AB serum should be added to it.
2. Add 1 drop of 3-5% suspension of washed O Rh (D) positive red cells to the serum in the tube.
3. Mix and incubate at 37°C for 30-40 minutes.
4. Centrifuge at 1000 rpm for 1 minutes.
5. Examine for hemolysis and/or agglutination. Use optical aid if necessary. Agglutination at this stage indicates the presence of saline (complete) antibodies.
6. If no agglutination is seen, wash cells 3-4 times in large volume of saline. Decant supernatant in each wash as completely as possible.

:Procedure

7. Add 2 drops of AHG serum to the cells.
8. Mix and centrifuge at 1000 rpm for 1 minutes immediately.
9. Gently shake the tube to dislodge the button and examine for agglutination, using optical aid. Record the result.
10. Add 1 drop of IgG coated red cells to any test that is negative. Mix and centrifuge at 1000 rpm for 1 minutes. Look for agglutination. If there is no agglutination, the test result is invalid and the whole test is repeated. If agglutination is obtained the result is valid.
11. Auto control should be kept with IAT





Serum/plasma

Screening cells x2/3/4

Incubation

Wash x3

Only bound antibody on RBC

Addition of AHG

Centrifugation → agglutination

Resuspend, read over light source

BOVINE ALBUMIN(22%)-IAT

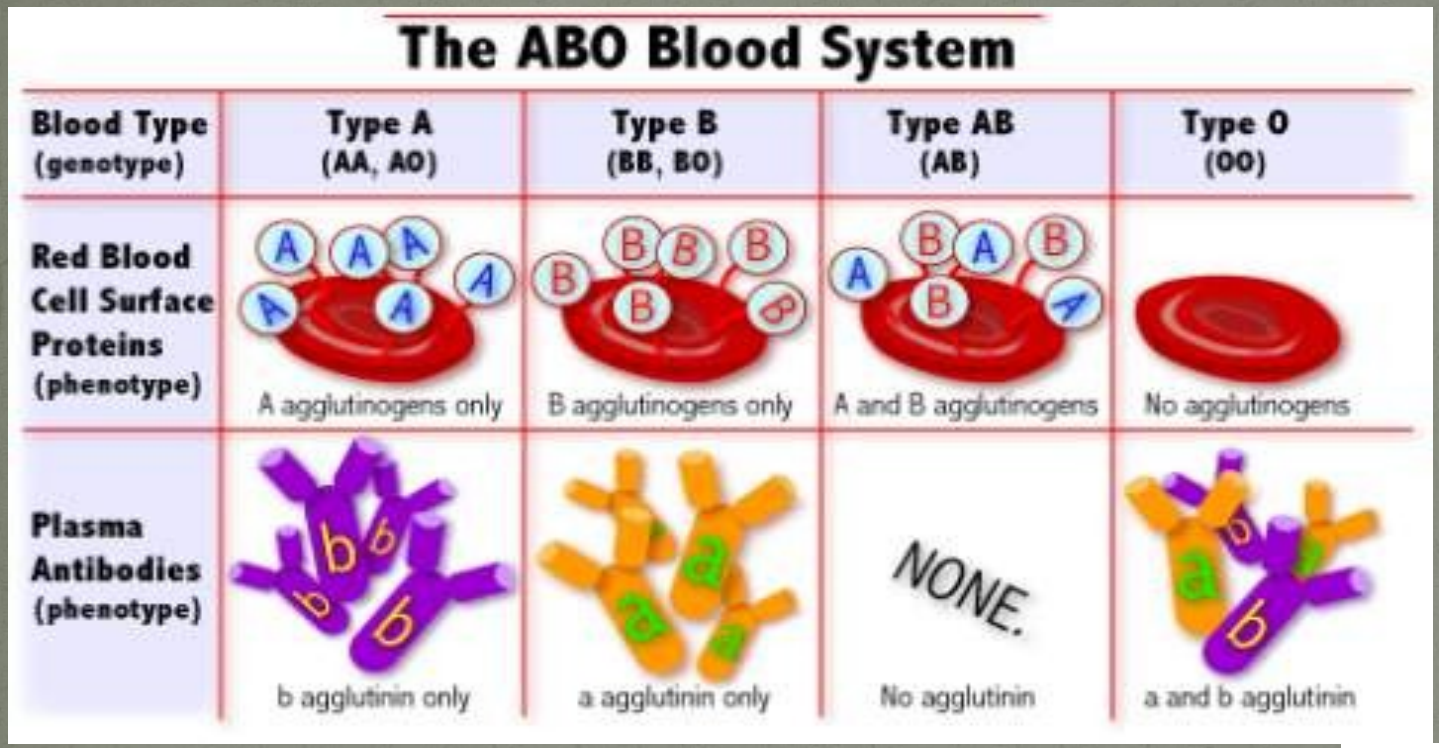
One Stage Method - Additive method

- Procedure:
  1. Two drops of albumin 22.5% are added in step (2) of saline-IAT
  2. Mix and incubate for 20-30 minutes at 37°C
  3. Proceed further as in saline-IAT procedure.

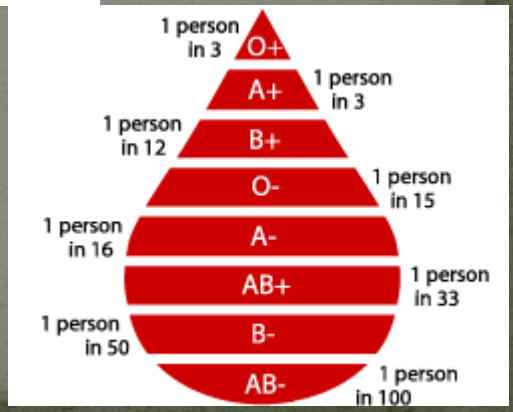
CBC Parameters

PARAMETER	UNIT OF REPORTING	COMMON METHOD OF DETERMINATION
WBC	X 10^3 /µL	Impedance count X calibration (cal) factor
RBC	X 10^6 /µL	Impedance count X calibration factor
HGB	g/dL	Colorimetric absorbance in proportion to hemoglobin
MCV	fL	, From RBC histogram of RBCs X size of RBCs X cal constant OR **Calculated:# $\frac{\text{HCT X 10}}{\text{RBC}}$**
HCT	%	**Calculated: $\frac{\text{RBC X MCV}}{10}$**
MCH	Pg	**Calculated: $\frac{\text{HGB X 10}}{\text{RBC}}$**
MCHC	%g/dL or	**Calculated: $\frac{\text{HGB X 100}}{\text{HCT}}$**
RDW	%	Impedance (from histogram)
Platelet	X 10^3 /µL	Impedance count X cal factor
WBC Diff	Absolute: X10^3 /µL : %Percent of WBC	Light Scatter , flow cytometry

Blood Types



http://www.bloodbook.com/world-abo.html



Blood Typing

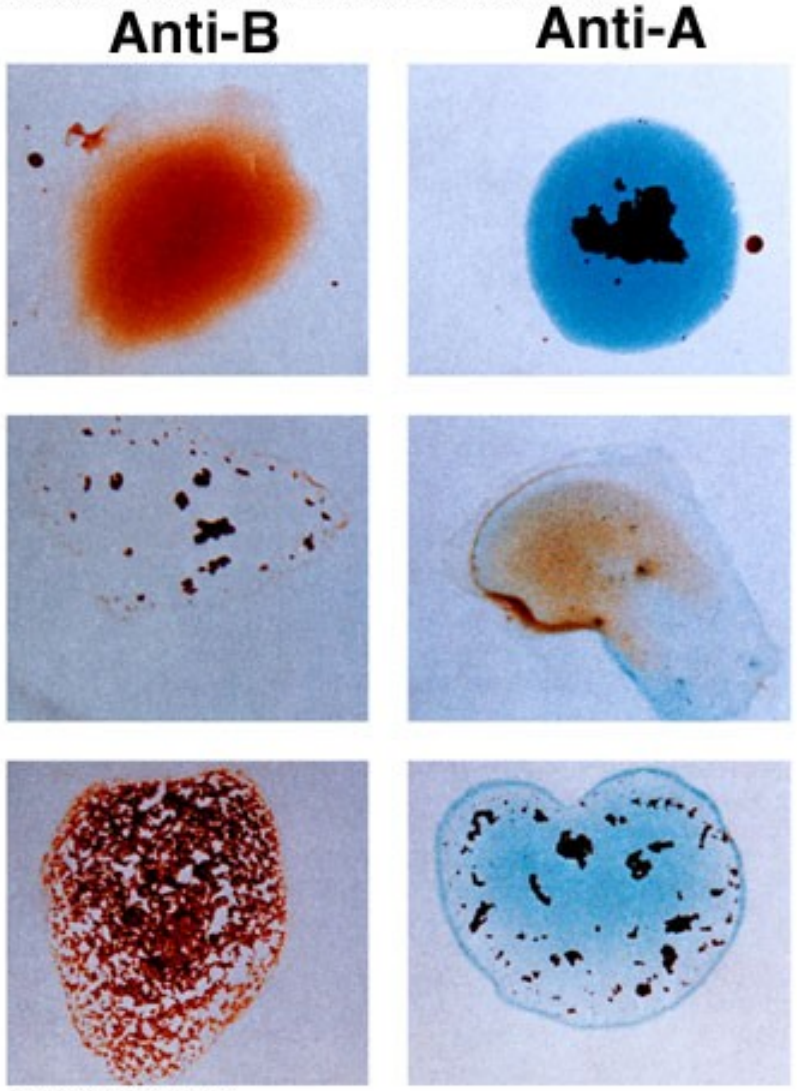
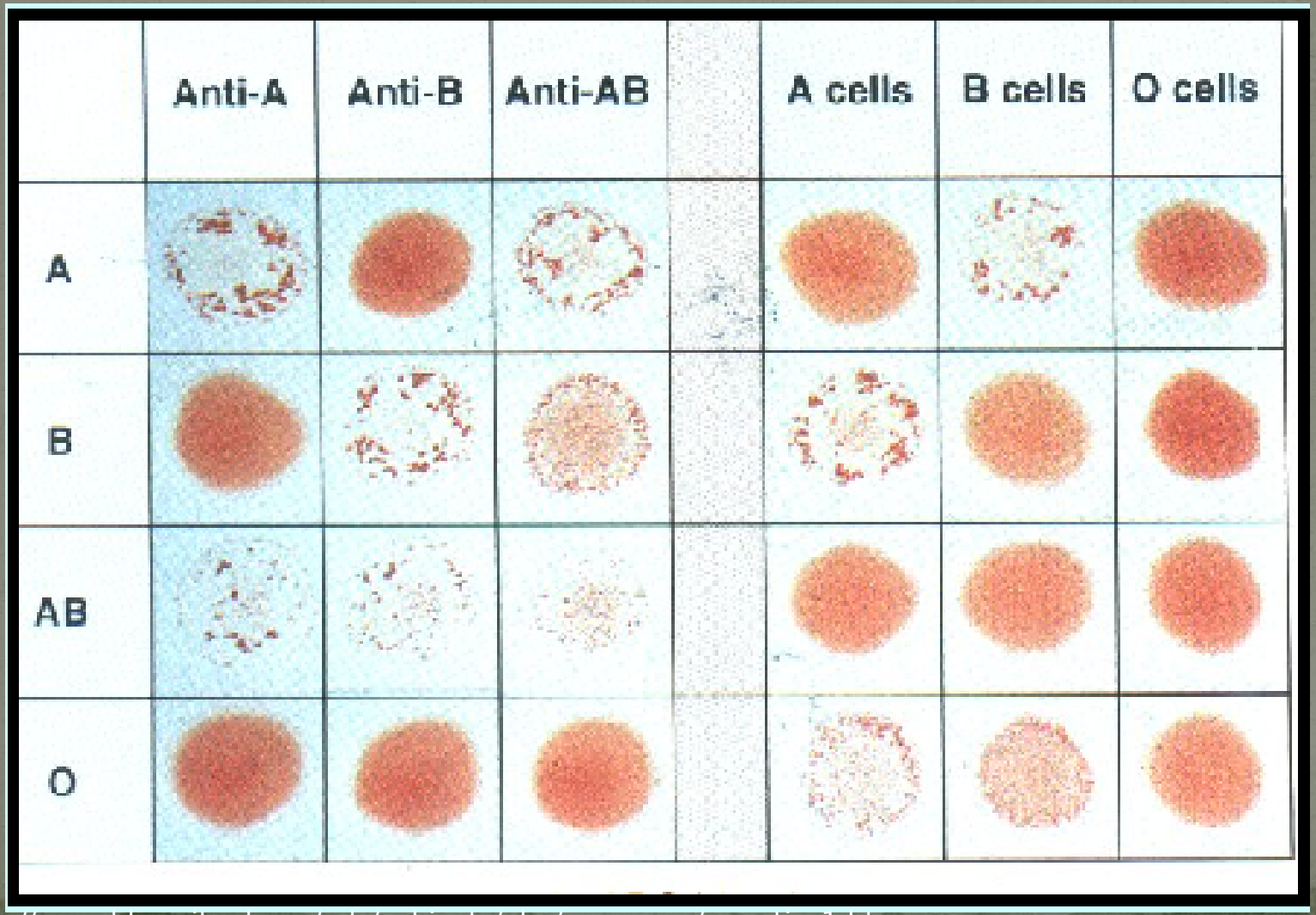


Illustration of the forward and reverse grouping reaction patterns of the ABO groups using a blood group tile



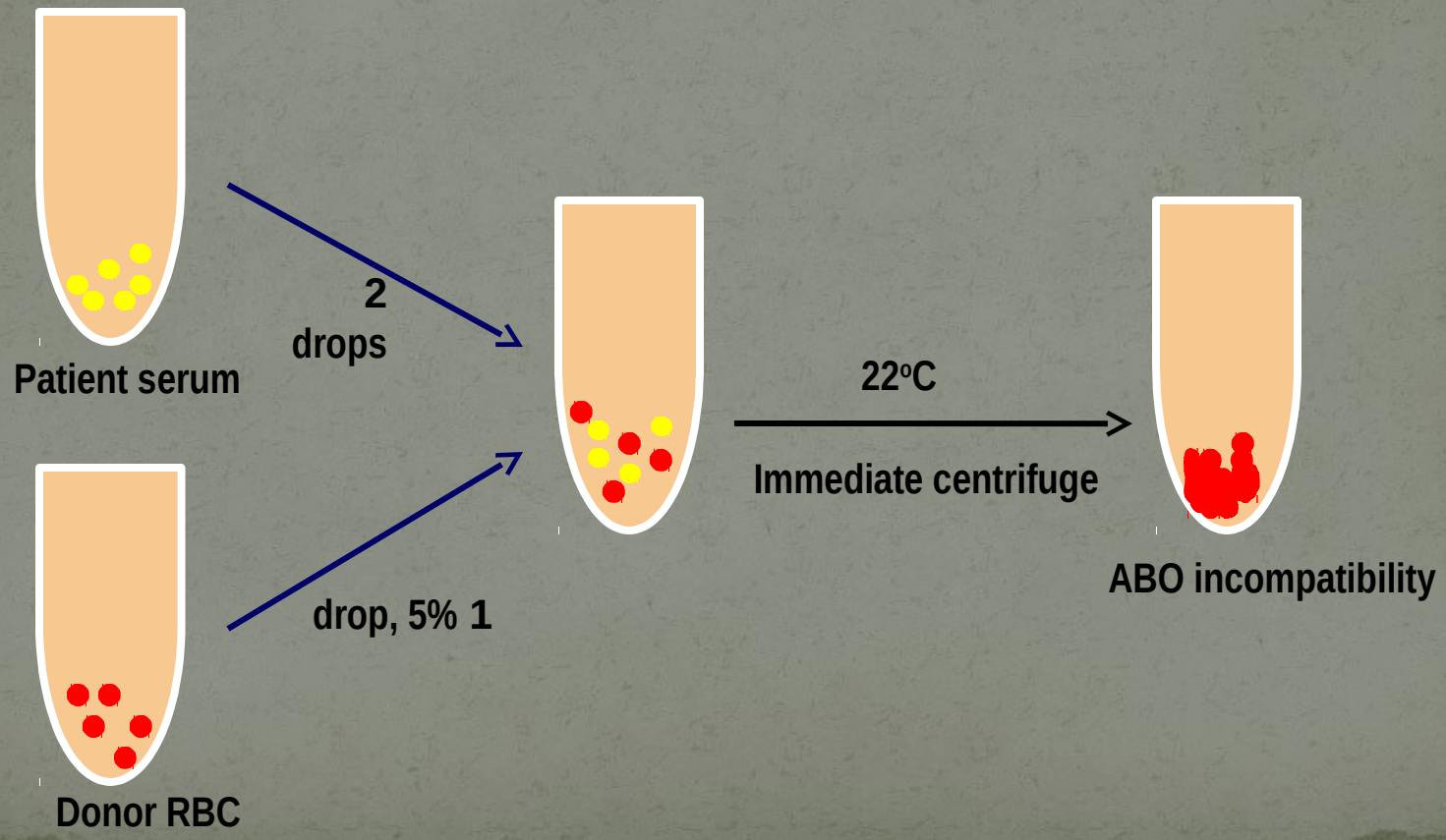
http://www.bh.rmit.edu.au/mls/subjects/abo/resources/genetics1.htm

Cross Matching Procedure

- Cross matching should be performed at following phases
  - Saline phase at room temperature
  - AHG phase
- Cross matching can be performed using conventional test tubes or by using newer technologies such as
  - Column Agglutination Technology
  - Solid Phase Technology
  - Electro Magnetic (EM) Technology

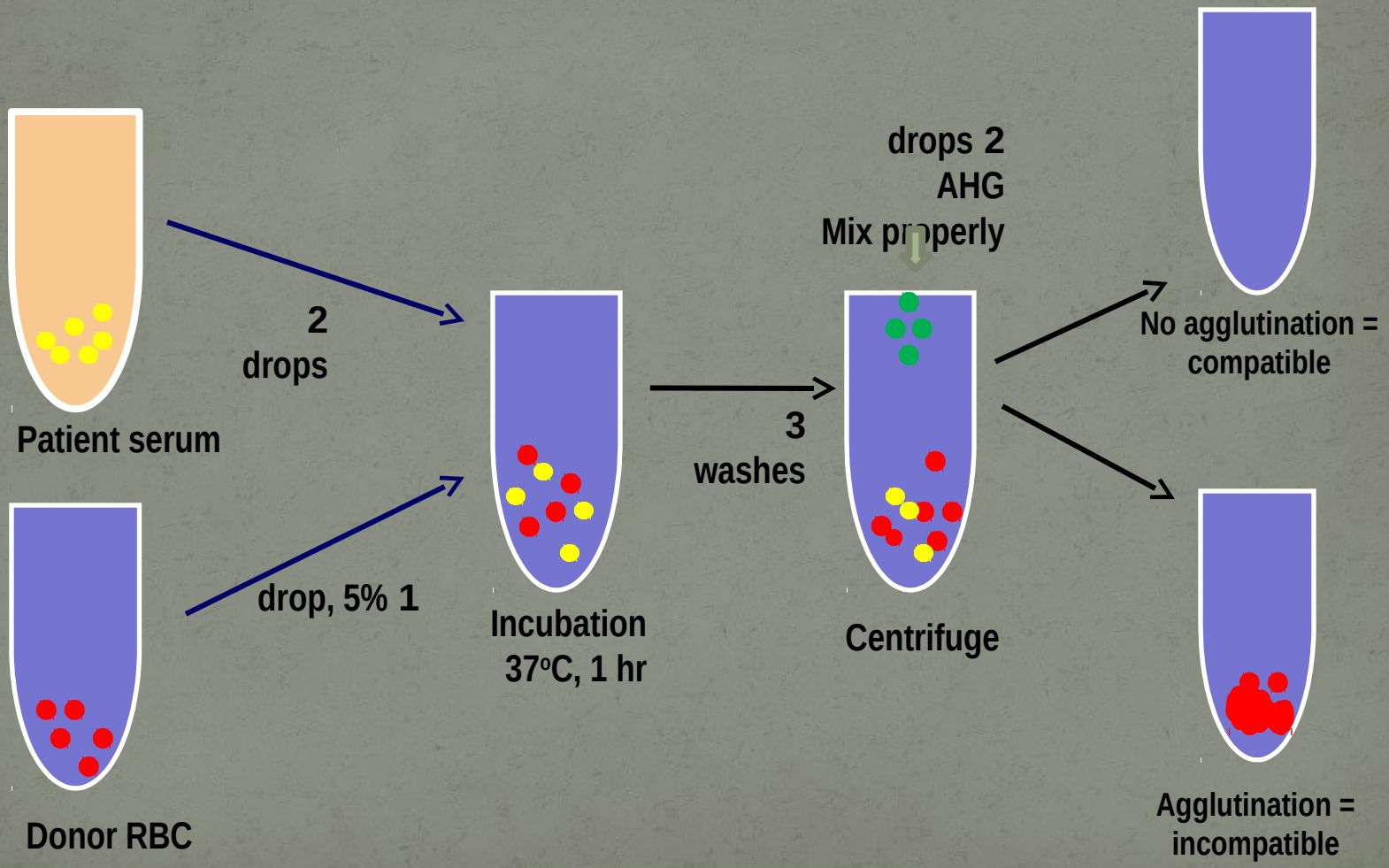
Immediate Spin Technique (IST)

- Detects only IgM antibody, reactive at 22°C.
- Clinically significant IgG antibody reactive at 37°C not detected



Conventional AHG-crossmatch

Detects clinically significant (IgG) antibody



Serological cross match

Phase	Detects
IS phase	ABO incompatibilities
AHG phase	Rh, Duffy, Kidd, others

Points to remember:

- ✓ Preserve recipients serum & donor red cell segment for a week.
- ✓ However, fresh sample of the patient is needed after 48 hrs of transfusion
- ✓ Do not withdraw sample from the IV line
- ✓ Infuse red blood cells within 4 hours

ABO Selection of Blood Components

Patient ABO Type	*RBCs, Platelets*	Plasma & Cryoprecipitate
O	O	O, A, B, AB
A	A,O	A,AB
B	B,O	B,AB
AB	AB,A,B,O	AB

Anticoagulants Preservative Solutions

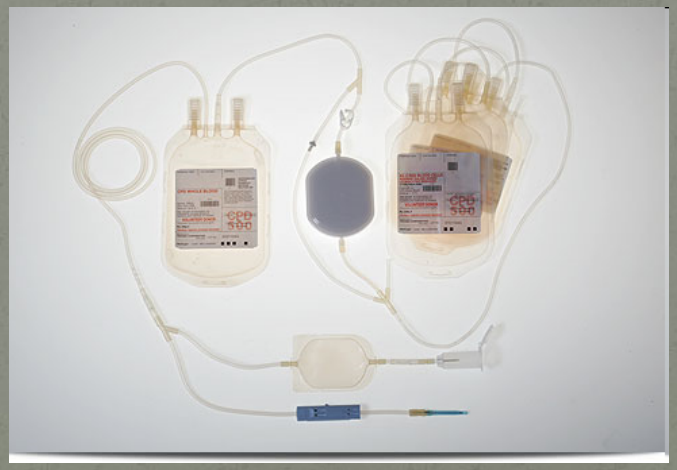
- Anticoagulants prevent blood clotting
- Preservatives provide nutrients for cells
- Heparin
  - Rarely if ever used anymore
  - Anticoagulant ONLY
  - Transfuse within 48 hours, preferably 8

Anticoagulants

	CPD or CP2-D	CPD-A1
Storage time	21 days	35 days
Temperature	1-6 C	1-6 C
	Slows glycolytic activity	
Adenine	None	Substrate for ATP synthesis
Volume	450 +/- 10%	
Dextrose	Supports ATP generation by glycolytic pathway	
Citrate	Prevents coagulation by binding	

Additive Solution (AS)

- Primary bag with satellite bags attached.
- One bag has additive solution (AS)
- Unit drawn into CPD anticoagulant



Additive Solution

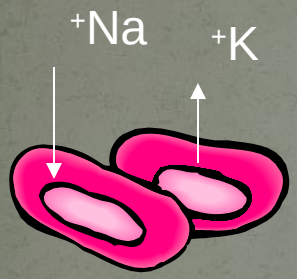
- Remove platelet rich plasma within 72 hours
- Add additive solution to RBCs, ADSOL, which consists of:
  - Saline
  - Adenine
  - Glucose
  - Mannitol
- Extends storage to 42 days
- Final hematocrit approximately 66%

Changes Occur During Storage

- Shelf life = expiration date
  - At end of expiration must have 75% recovery
  - At least 75% of transfused cells remain in circulation 24 hours AFTER transfusion

Storage Lesion

- Biochemical changes which occur at 1-6C
- Affects oxygen dissociation curve, increased affinity of hemoglobin for oxygen.
  - Low 2,3-DPG, increased O_2 affinity, less O_2 released.
  - pH drops causes 2,3-DPG levels to fall
  - Once transfused RBCs regenerate ATP and 2,3-DPG
- Few functional platelets present
- Viable (living) RBCs decrease



Red Blood Cell

Plasma hemoglobin

+Plasma K

Viable cells

pH

ATP

DPG-2,3

+Plasma Na

Helps release oxygen from hemoglobin (once transfused, ATP & 2,3-DPG return to normal)

Storage Lesion

- Significant for infants and massive transfusion.
- Summary of biochemical changes
  - pH decreases
  - 2,3 DPG decreases
  - ATP decreases
  - Potassium increases
  - Sodium decreases
  - Plasma hemoglobin increases

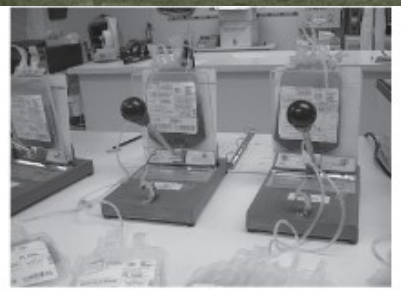
Preparation of Components

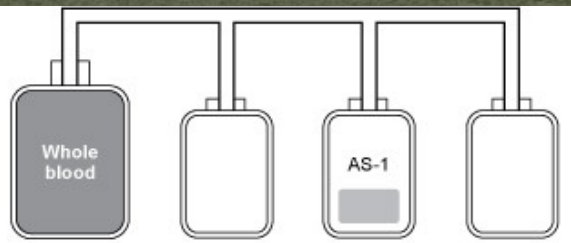
- Collect unit within 15 minutes to prevent activation of coagulation system
- Draw into closed system – primary bag with satellite bags with hermetic seal between.
- If hermetic seal broken transfuse within 24 hours if stored at 1-4C, 4 hours if stored at 20-24C

Preparation of Components

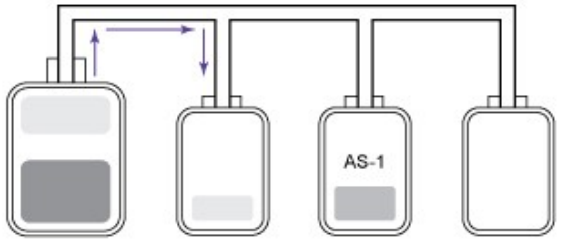
- Centrifuge – light spin, platelets suspended
- Remove platelet rich plasma (PRP)
- Centrifuge PRP heavy spin
- Remove platelet poor plasma
- Freeze plasma solid within 8 hours
- Thaw plasma at 1-4C – precipitate forms
- Centrifuge, express plasma leaving cryoprecipitate. Store both at -18C
- RBCs – CPD – 21 days, ADSOL – 42 days – 1-6C

A

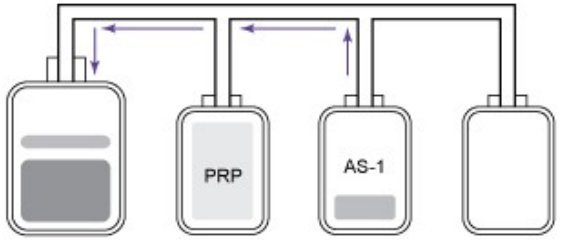




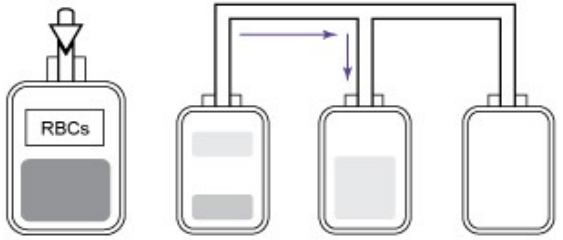
Whole blood with satellite bags attached is centrifuged at a light spin.



Platelet-Rich Plasma is expressed off the Red Blood Cells into a satellite bag.



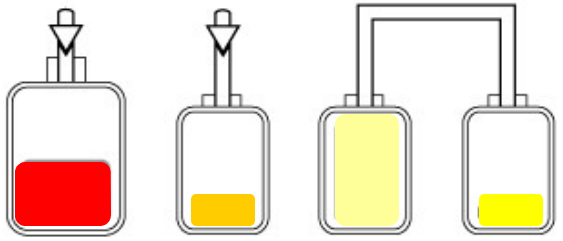
The additive solution is added to the Red Blood Cells.



Red Blood Cells are sealed and cut off.

Platelet-Rich Plasma is centrifuged.

Plasma is expressed off the platelets.



The platelets are sealed and the tubing cut.

The plasma is either frozen to make Fresh Frozen Plasma or frozen and thawed to make Cryoprecipitated Antihemophilic Factor

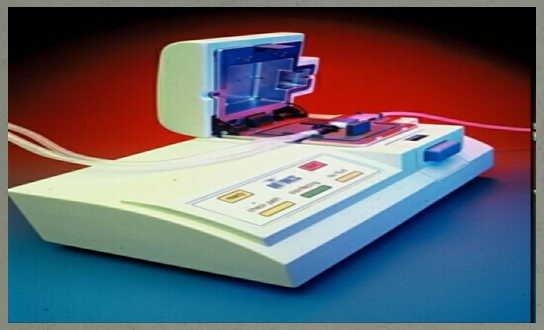
B Red Blood Cells Platelets FFP CRYO

A, Courtesy LifeSouth Community Blood Centers, Gainesville, Fla.

Preparation of Components

- Summary – One unit of whole blood can produce:
  - Packed RBCs
  - Fresh frozen plasma (FFP)
  - Cryoprecipitate (CRYO)
  - Single donor plasma (SDP) – cyro removed
  - Platelets

Preparation of Components



- Sterile docking device joins tubing
  - Used to add satellite bags to maintain original expiration of component
  - May be used to pool components

Blood Component General Information

- Blood separated into components to specifically treat patients with product needed
- Advantages of component separation
  - Allow optimum survival of each component
  - Transfuse only component needed

Blood Component General Information

- Transfusion practice
  - Transfusion requires doctor’s prescription
  - All components MUST be administered through a filter
  - Infuse quickly, within 4 hours
  - D (Rh) neg require D neg cellular products
  - ABO identical preferred, ABO compatible OK
  - “Universal donor” – RBCs group O, plasma AB

Blood Component General Information

- Fresh Whole Blood
  - Blood not usually available until 12-24 hours
  - Candidates
    - Newborns needing exchange transfusion
    - Patients requiring leukoreduced products – in US products leukoreduced immediately after collection.

Blood Component General Information

- Summary of storage temperatures:
  - Liquid RBCs 1-6C
  - Platelets, Cryo (thawed) and granulocytes 20-24C (room temperature)
  - ANY frozen plasma product ≤ -18C
  - ANY liquid plasma product EXCEPT Cryo 1-6C

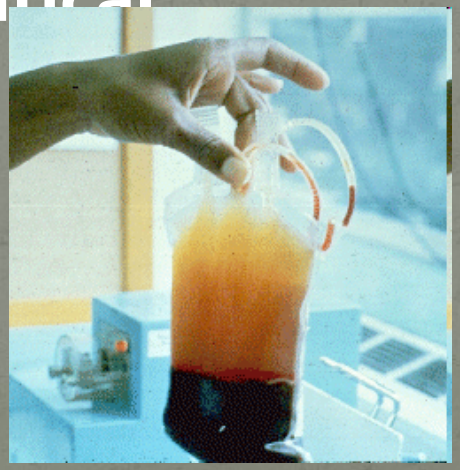
Blood Components

- Cellular
  - Red blood cell products
  - Platelets
  - Granulocytes
- Plasma
  - FFP
  - Cryoprecipitate

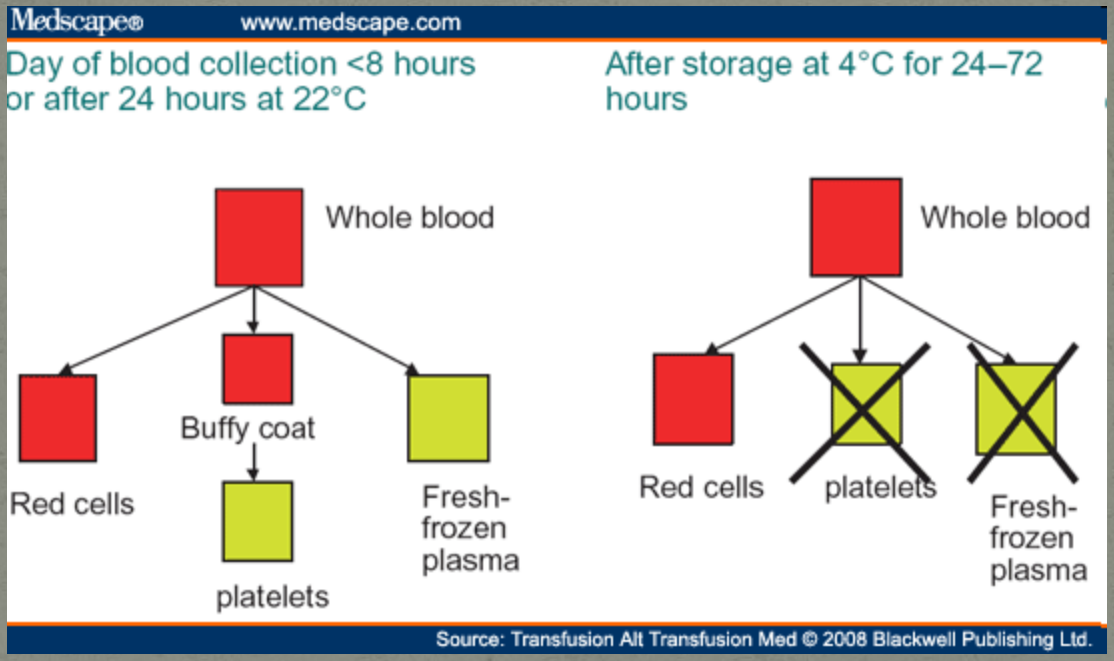
Products With Red Cells

Whole Blood

- Clinical indications for use of WB are **extremely** limited.
- Used for massive transfusion to correct acute hypovolemia such as in trauma and shock, exchange transfusion.
- RARELY used today, platelets non-functional, labile coagulation factors gone.
- **Must** be ABO **identical**



Changes in Stored Blood



Red Blood Cells (RBC)

- Used to treat symptomatic anemia and routine blood loss during surgery
- Hematocrit is approximately 80% for non-additive (CPD), 60% for additive (ADSOL).
- Allow WB to sediment or centrifuge WB, remove supernatant plasma.

Cell Washer Prepares Washed Cells

Thank you