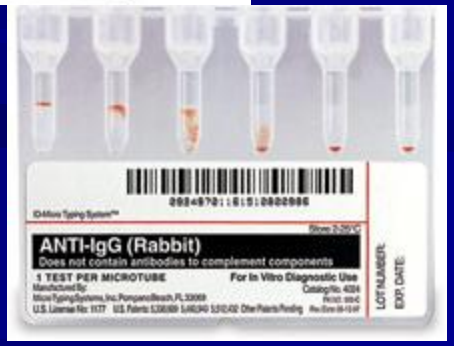
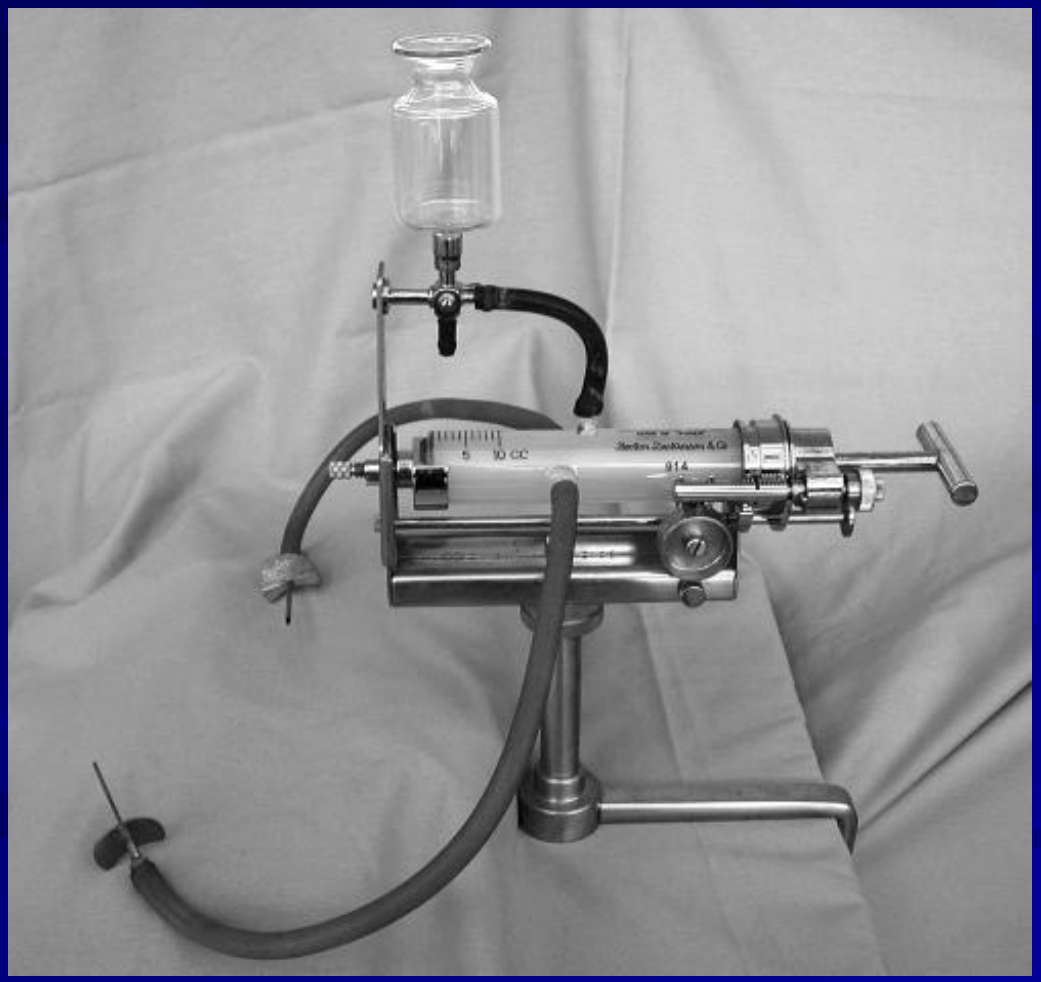
Pretransfusion / Compatibility testing



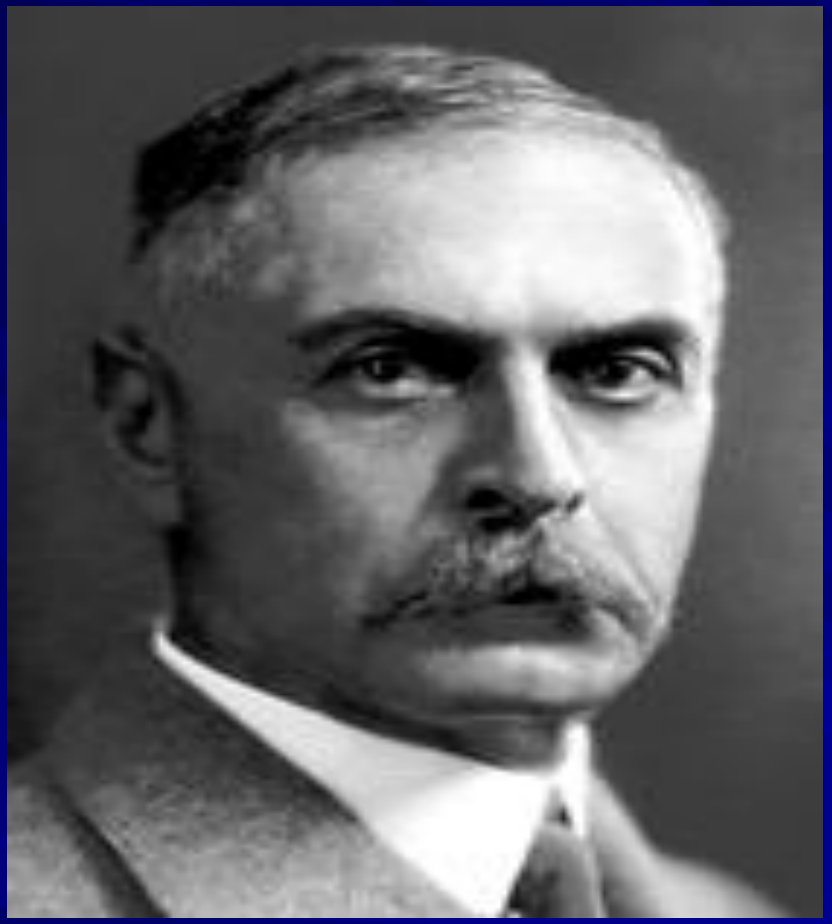
"Medical Center" instrument for direct transfusion



Transfusion
Volume 46 Page 497 - April 2006

World War II syringe for direct interhuman blood transfusion

Karl Landsteiner



Did a lot of work in:

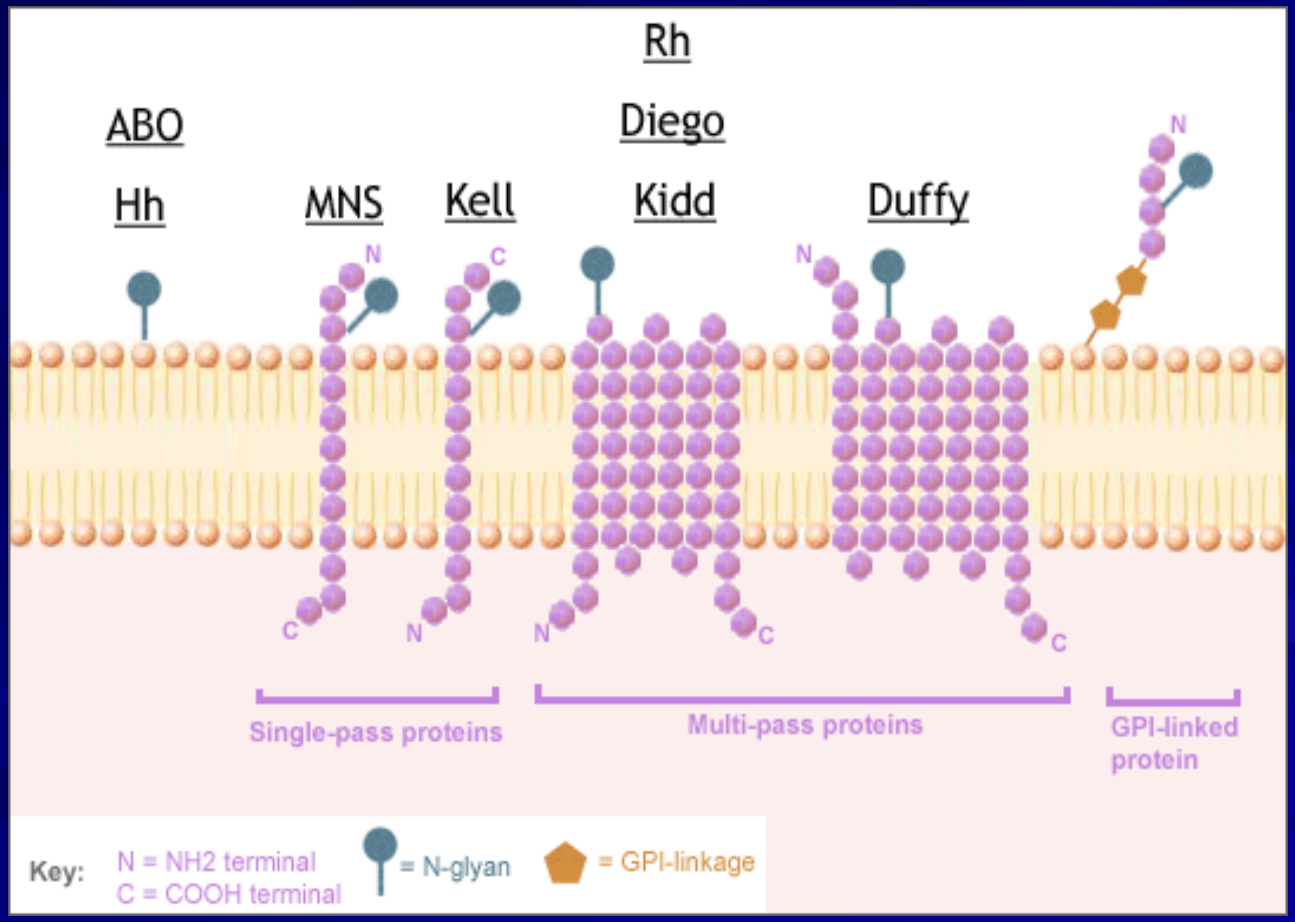
- **Syphilis**
- **Haptens**
- **PCH**
- **Polio**
- **ABO blood groups**
- **Died in 1943 after a heart attack in his lab at Rockefeller institute**

http://nobelprize.org/medicine/laureates/1930/landsteiner-bio.html

Blood Group Antigens

- Inherited, different genes encode for different antigens
- Codominant
- Determine an individuals blood group
- Function of the different antigens unclear
  - Receptor for certain bacteria, viruses and parasites
  - Cytokine receptors

Blood group antigens

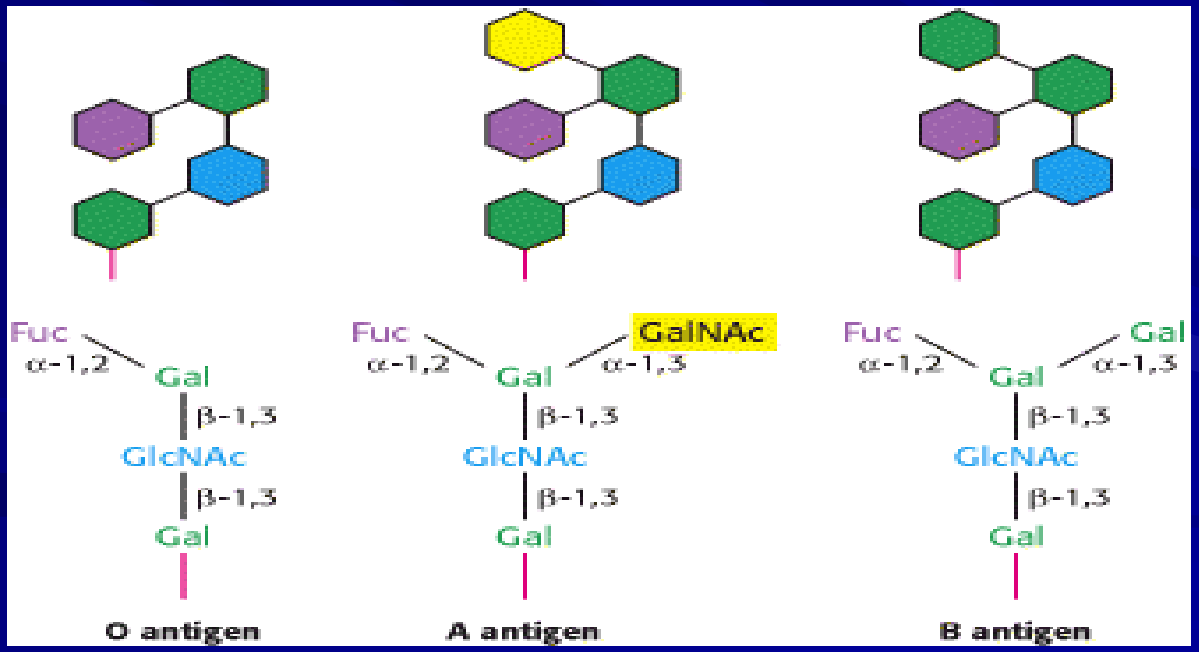


Blood group antigens are either sugars or proteins, and they are attached to various components in the red blood cell membrane

http://www.ncbi.nlm.nih.gov

ABO Blood Group System

Most important, highly immunogenic blood group found on red blood cells and other tissue cells



http://www.ncbi.nlm.nih.gov/books

ABO blood groups

- Genes Controlling ABO groups
  - H gene - H substance
  - A gene - A enzyme
  - B gene - B enzyme
  - O gene - amorphic gene, no enzyme

Formation of the A, B, and H antigen

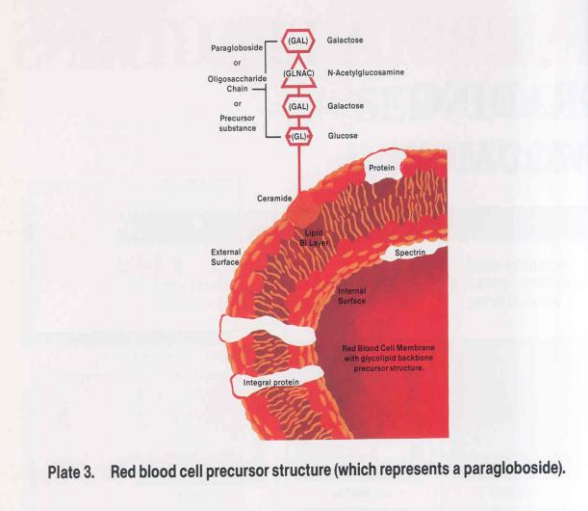


Plate 3. Red blood cell precursor structure (which represents a paragloboside).

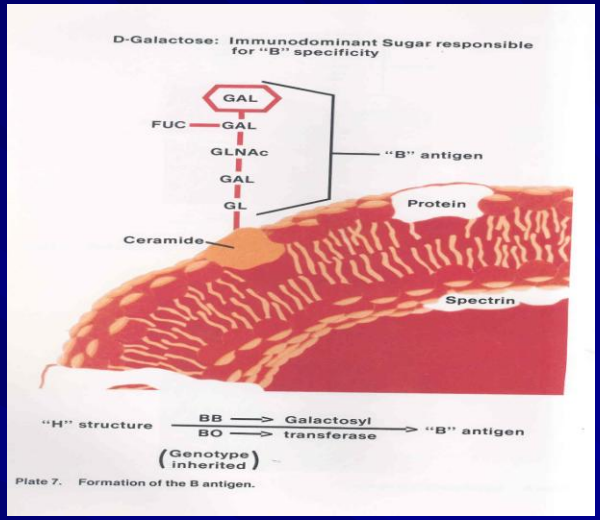


Plate 7. Formation of the B antigen.

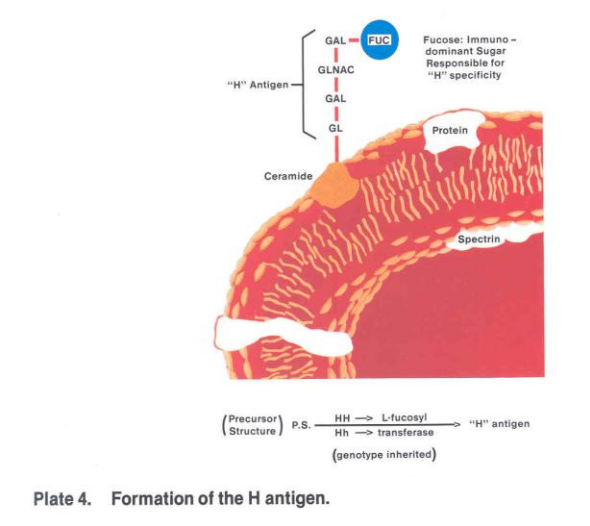


Plate 4. Formation of the H antigen.

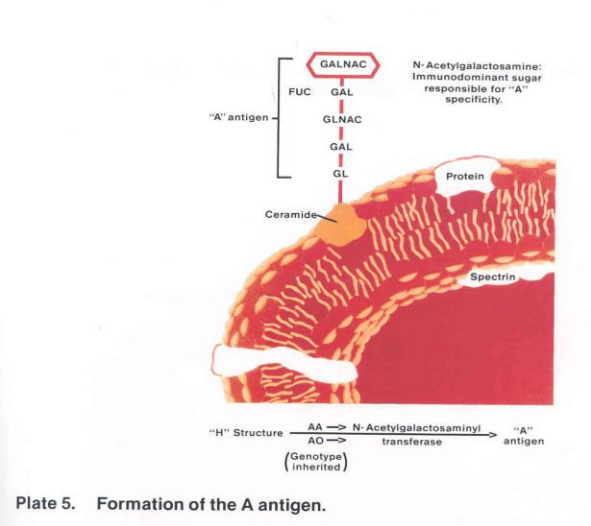


Plate 5. Formation of the A antigen.

From: Harmening. Modern Blood Banking and Transfusion Practices

ABO Genotypes, Phenotypes and Frequencies

ABO phenotype	Genotype	Antigen	Frequency %
O	OO	Neither	45
A	AA or AO	A	41
B	BB or BO	B	10
AB	AB	A & B	4

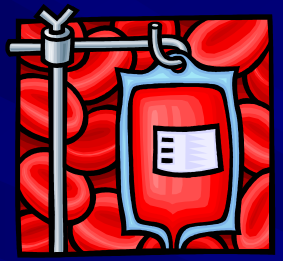
Blood group	Antigen(s) present on the red blood cells	Antibodies present in the serum	Genotype(s)
A	A antigen	Anti-B	AA or AO
B	B antigen	Anti-A	BB or BO
AB	A antigen and B antigen	None	AB
O	None	Anti-A and Anti-B	OO

ABO antibodies

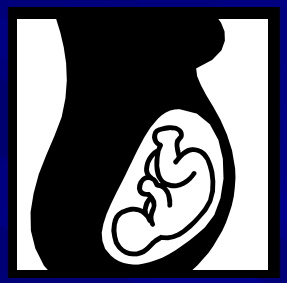
- detectable by age 3 months
- naturally occurring
- IgM antibodies; bind C3 - intravascular hemolysis
  - AB- no antibody
  - A - anti B
  - B - anti A
  - O -anti A, anti B, anti A,B

Antibody response

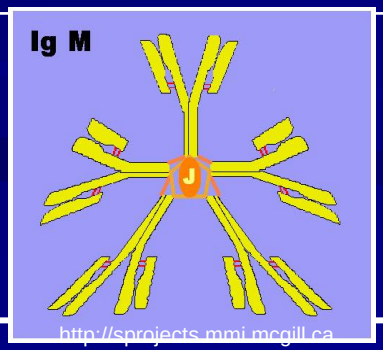
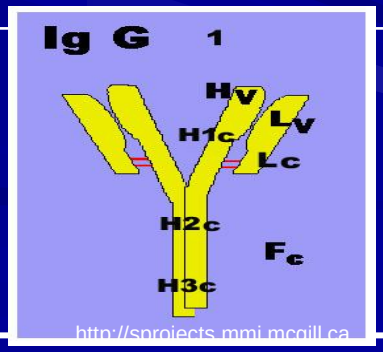
- Can occur on exposure to a foreign red cell antigen
  - Transfusion



  - Pregnancy



- Depends on “immunogenecity” of antigen
  - ABO and Rh group most immunogenic
- Naturally occurring antibodies can form

IgM	IgG
Naturally occurring	Immune stimulated
React at “cold” temperatures	React at “warm” temperatures
No red cell hemolysis	Red cell hemolysis
Does not cross the placenta	Can cross the placenta
	

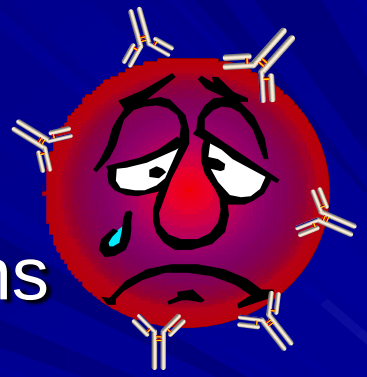
Blood Selection

- donor
  - identification of donor
  - testing of donor
- recipient
  - identification of recipient
  - review of transfusion history
  - compatibility testing
  - selection of appropriate donor units
  - identification of patient before infusion of blood

Goal

- Ensure maximum red cell survival
  - acceptable survival of donor rbcs

  - no destruction of recipient's rbcs
  - Prevent hemolytic transfusion reactions

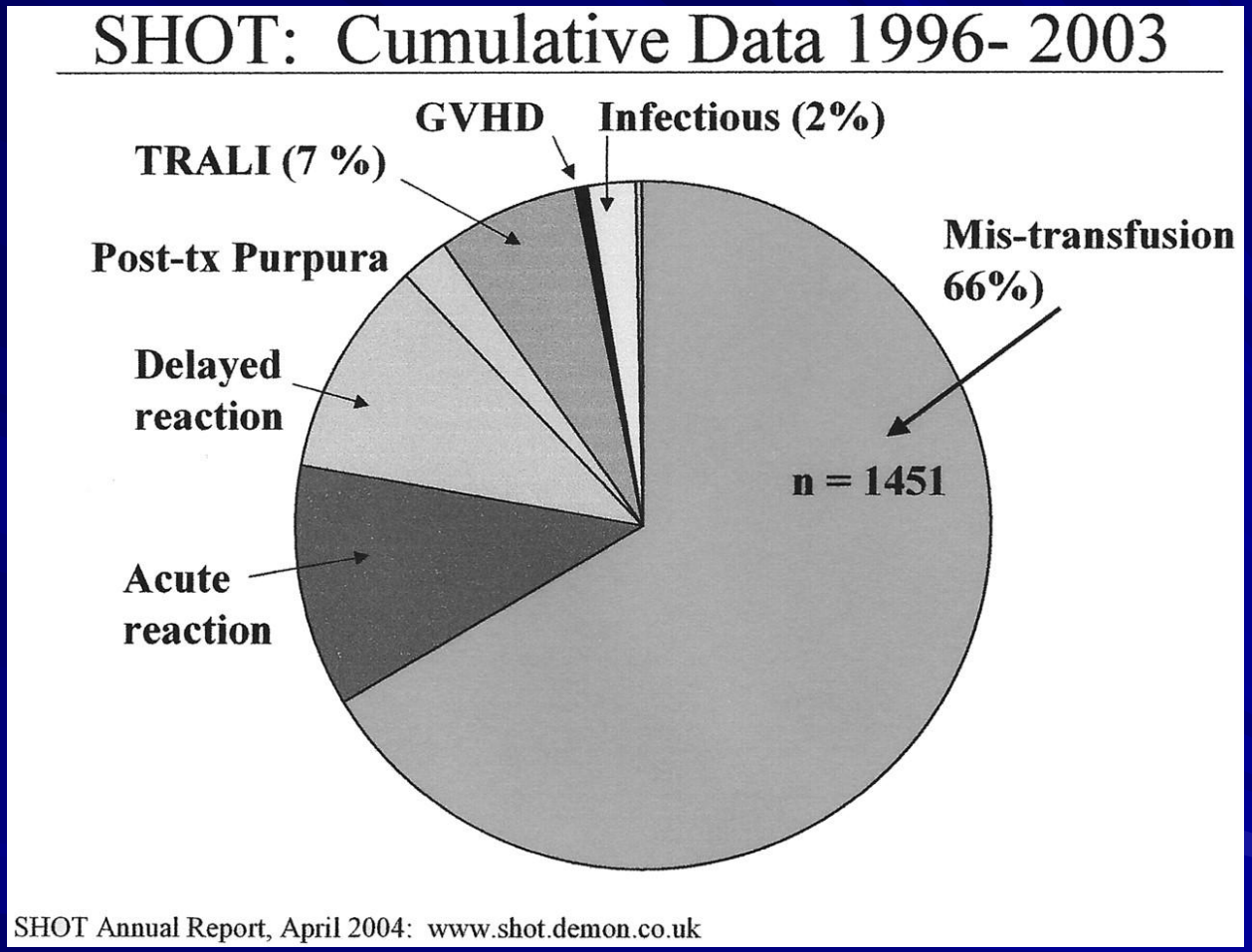


- Prevent disease transmission

Hemolytic transfusion reactions

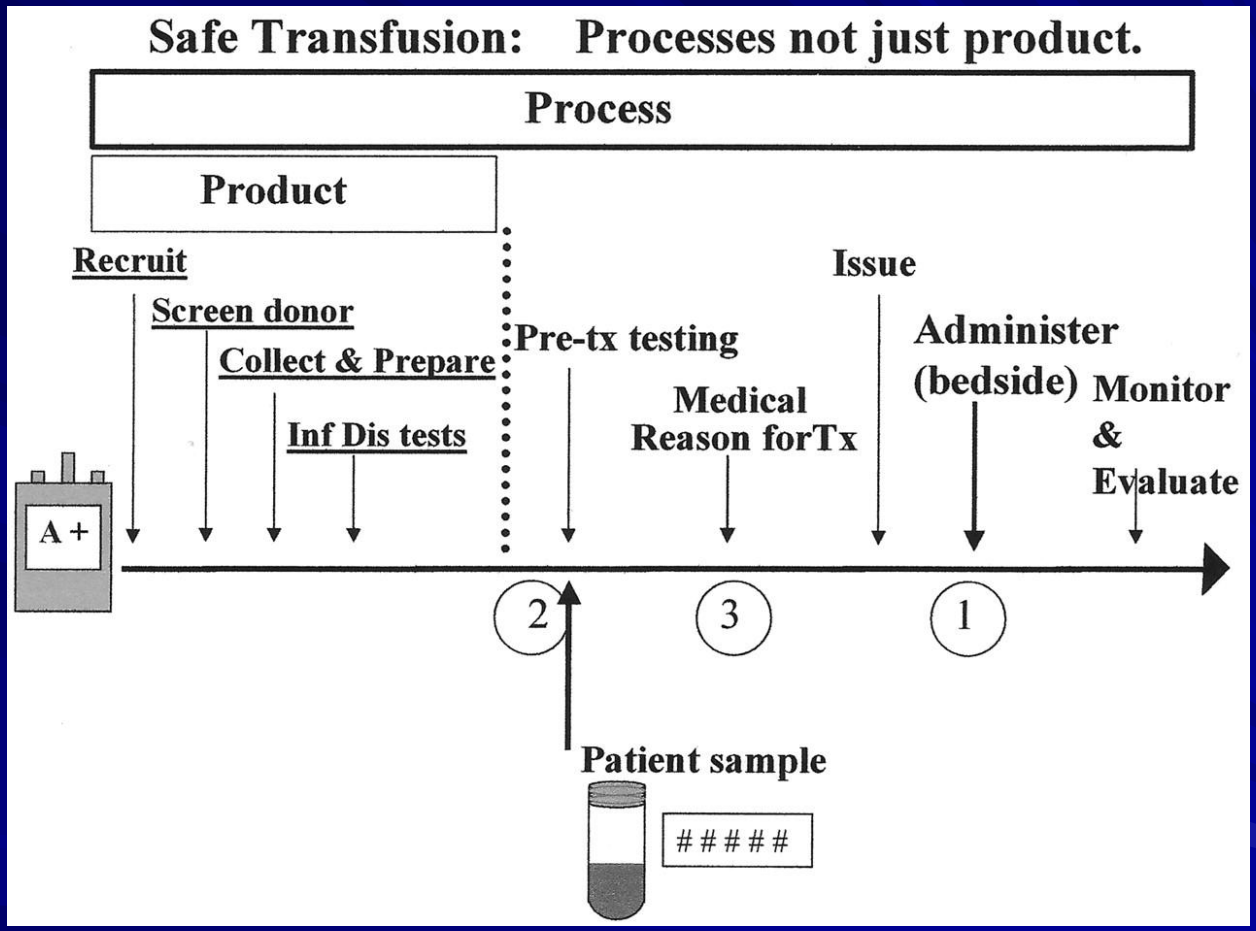
- Clerical and other human errors are the most common causes of ABO incompatible transfusions
  - Preanalytical
  - Analytical
  - Post analytical

Fatal Adverse Events in the United Kingdom



Dzik, W. H. Hematology 2005;2005:476-482

Safe transfusion from donor to recipient



Dzik, W. H. Hematology 2005;2005:476-482

Pre-transfusion testing

- Compatibility testing
  - ABO blood group and D typing.
  - Antibody screen and identification.
  - Cross-match.
  - DAT

- **Detection of platelet antibodies.**
- **WBC quantitation in leukoreduced products.**
- **Feto-maternal hemorrhage.**

AABB requirements

- Positive identification of recipient and recipient blood sample

Mismatched Blood Kills Patient at Inova Fairfax
Washington Post (08/29/03) P. B1

At Inova Fairfax Hospital, a patient was given the wrong blood type during surgery after she had switched beds with her roommate to be closer to the window. **The blood technician had withdrawn blood from the patient's roommate and failed to verify that the roommate was the correct patient**. Technicians are required to check the patient's name, clearly marked on his/her hospital bracelet, or ask the patient to state their name aloud. During the intestinal surgery, the deceased patient was given two pints of the wrong blood, causing her immune system to attack the donated cells--reducing her blood pressure, causing kidney failure, and prompting an acute hemolytic transfusion reaction. Doctors tried desperately to save the patient, but she died shortly after. An internal probe of the incident has prompted the hospital to have two technicians visit patients when blood is withdrawn. However, the family could possibly sue the hospital for malpractice and negligence; the technician has since resigned her post.

AABB requirements contd.

- ABO group and Rh typing of recipient's blood
- Red cell antibody detection tests for clinically significant antibodies
- Comparison of current findings with records
- Confirmation of ABO group of the red cell components
- Confirmation of the Rh type of the Rh negative units
- Selection of ABO and Rh appropiate components
- Serologic or computer crossmatch
- Labeling products with the recipient's identifying information
- Dispensing and administering the unit to the patient

Patient id and sample labelling

- ID patient in a positive manner
  - State name, birth date or address
  - Wristband
  - Blood bank number
  - Drivers license or other photographic id
- Label tube before leaving patient with identifiers, date of collection and id of phlebotomist

Transfusion requests

- Electronic or paper
- Requires
  - Two patient identifiers which should include first and last name, unique id #, DOB
  - Component needed
  - Special requests
  - Other clinical information
  - Name of responsible physician
- All blood banks should have a written policy defining the request acceptance criteria

Blood Orders

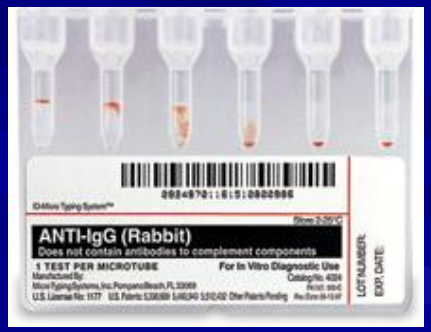
- Type
- Type and Screen
- Type and Hold
- Type and Crossmatch

Blood Sample

- Collected in EDTA tube
- Prefer non hemolyzed sample
- Sample should be collected no more than 3 days from intended transfusion
  - To ensure that current sample represents the current immunologic status of the patient
  - Retain for at least 7 days after each transfusion

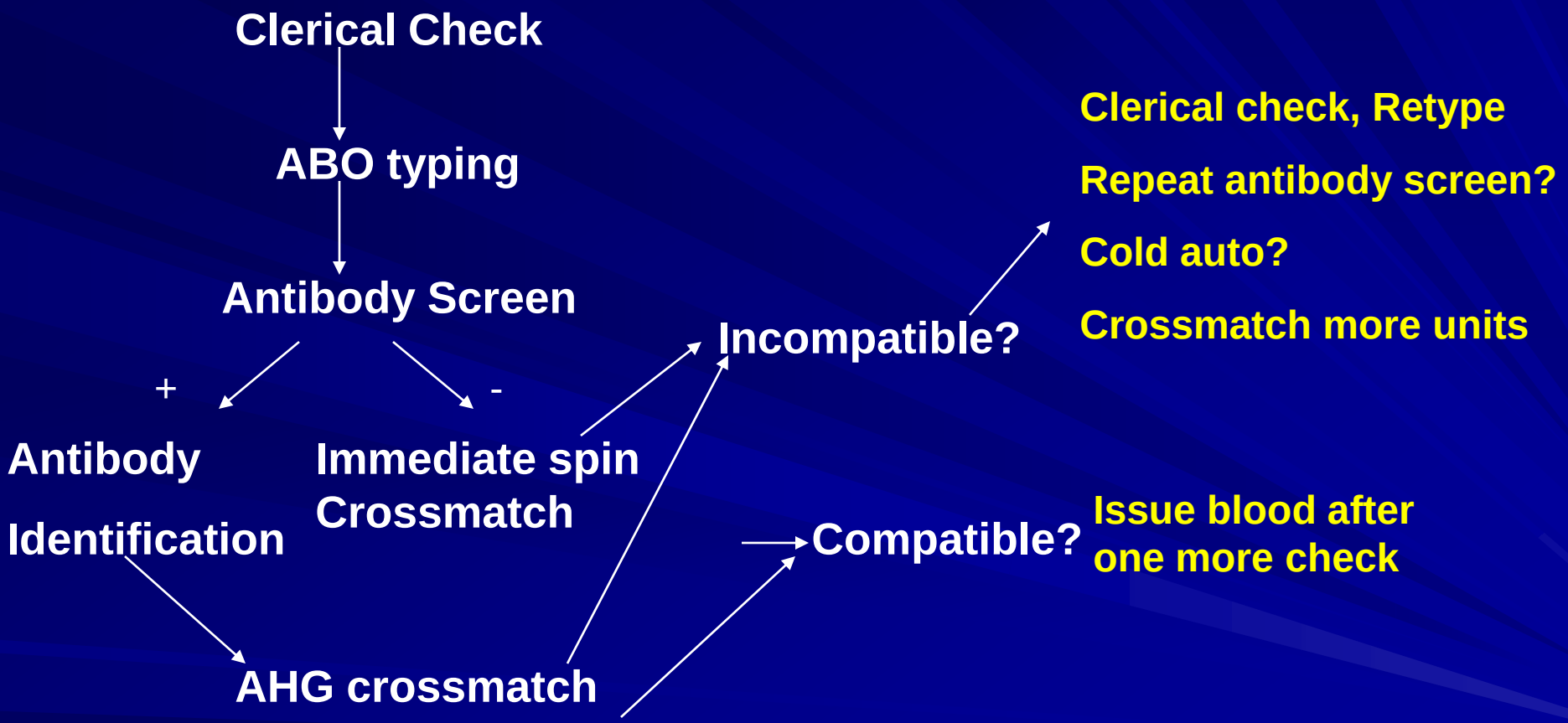
Serologic Testing

- Tests detecting antigen on recipient cells
  - ABO grouping (most critical test)
  - Rh typing
- Tests detecting antibody in recipient serum
  - Antibody screen
  - antibody identification
  - crossmatch



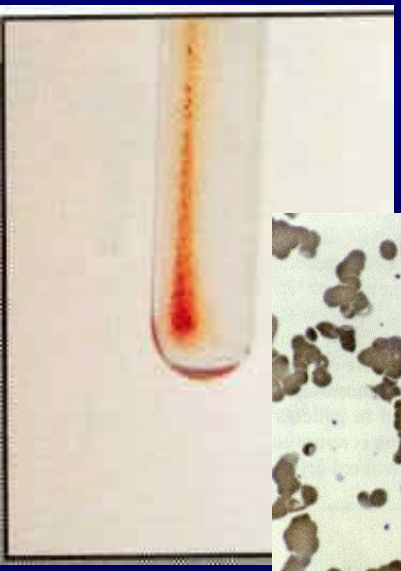
- Tests detecting Antibody on patient red cells
- Other tests

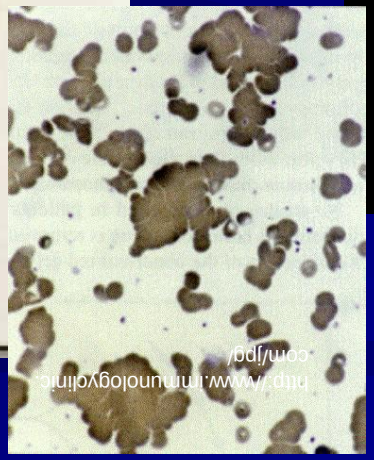
Pretransfusion testing algorithm



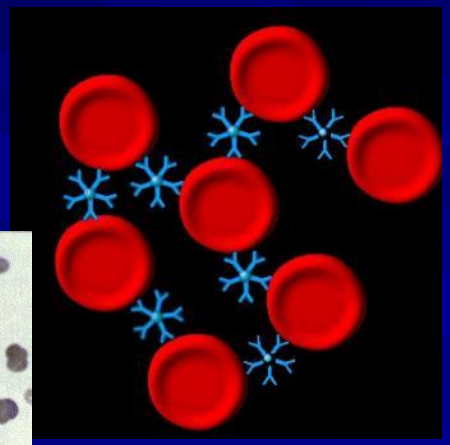
ABO Typing

- ***ABO typing is done by testing the patient's red cells with anti-A and anti-B antisera (forward grouping) and testing the patient's serum for the presence of anti-A and anti-B against reagent test A1 and B cells (reverse grouping). This test is done at room temperature and a positive reaction is determined by agglutination of the red cells.***





agglutination



IgM leads to the agglutination of red blood cells. Source: Med4you

reagents

Commercial monoclonal reagents available

Tube, slide, gel, microwell methods available

Tube most commonly used

Forward and Reverse Grouping

	Patient cells			**Patient serum**		
Patient	*Anti A*	*Anti B*	*Interpretation: Forward grp*	*A1 cells*	*B cells*	*Interpretation Reverse grp*
1	-	-	**O**	+	+	**O**
2	+	-	**A**	-	+	**A**
3	-	+	**B**	+	-	**B**
4	+	+	**AB**	-	-	**AB**

Rh Testing

- 85% of population are positive for the D antigen present on Rh molecule)
- D antigen is highly immunogenic
- antibody results following exposure
- IgG antibody
- important cause of hemolytic disease of the newborn

Antibodies produced against Rh antigens

Antibody type	**Mainly IgG, some IgM** The majority of Rh antibodies are of the IgG type.
Antibody reactivity	**Capable of hemolysis** Rh antibodies rarely activate complement. They bind to RBCs and mark them up for destruction in the spleen (extravascular hemolysis).
Transfusion reaction	**Yes typically delayed hemolytic transfusion reactions** Anti-D, anti-C, anti-e, and anti-c can cause severe hemolytic transfusion reactions. Hemolysis is typically extravascular
Hemolytic disease of the newborn	**Yes the most common cause of HDN.** **The D antigen accounts for 50% of maternal alloimmunization** **Anti-D and anti-c can cause severe disease.** **Anti-C, anti-E, and anti-e can cause mild to moderate disease.**

Rh Testing

- is done using anti D blood grouping serum

- tube or slide method
- Weak D
  - Decreased D antigen
  - Partial expression of D antigen
  - Can confirm by AHG testing

Antibody screen

- To detect as many clinically significant red cell antibodies as possible
  - antibodies reactive at 37^0C
  - known to cause transfusion reactions or shortened red cell survival
  - incidence 0.78 - 1.64%

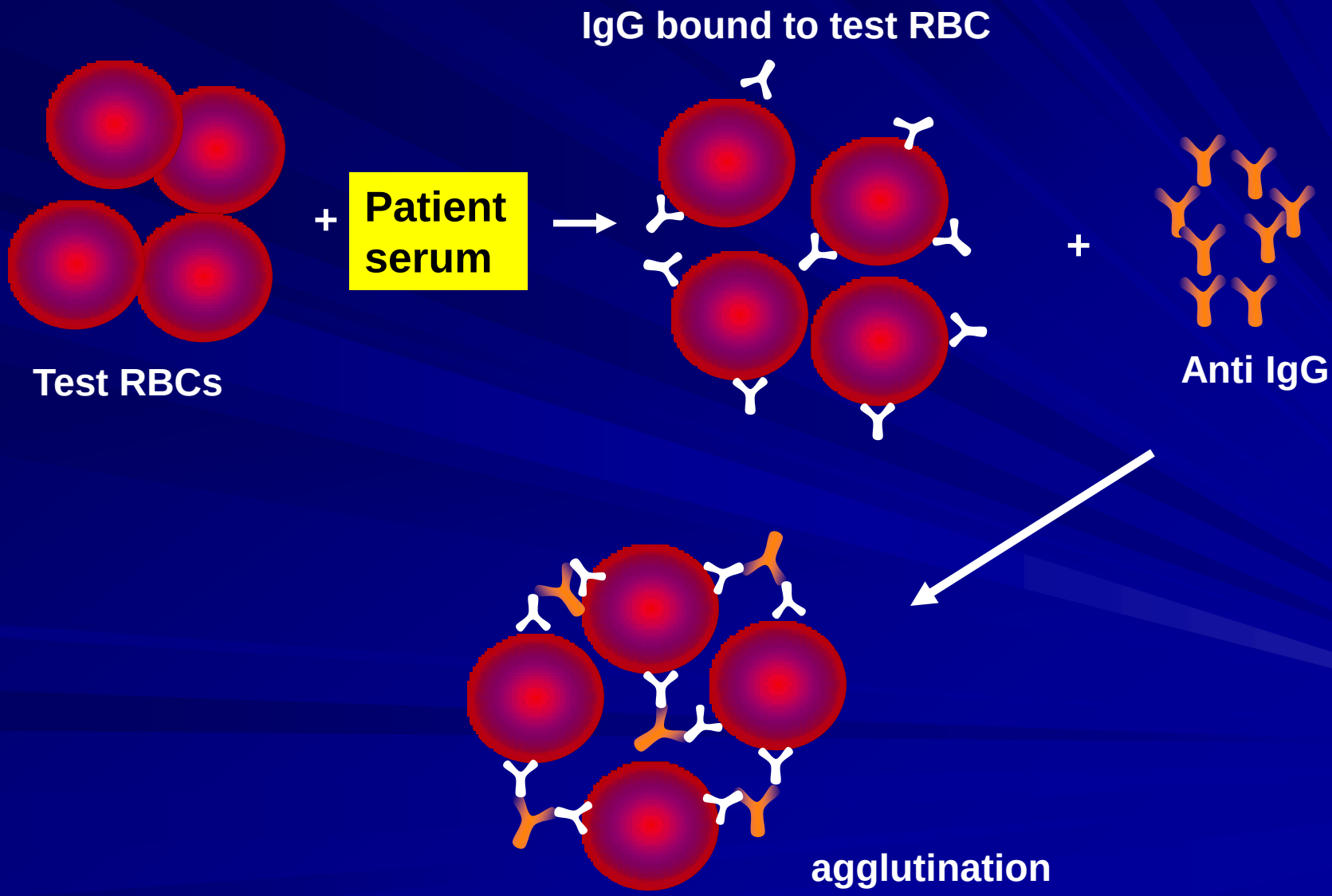
Antibody Screen

- is performed using selected group O rbcs that carry most of the common red cell antigens
- Testing on pooled cells not recommended
  - Only reading at AHG phase is required
  - Tube, gel, red cell solid phase
  - Enhancement media may be used
  - Use method that detects most clinically significant and few insignificant antibodies in a timely manner

Indirect antiglobulin test (IAT)

- Detects IgG antibodies in the patient's serum
- This is the methodology behind the antibody screen or identification

Indirect antiglobulin test



Gel Test

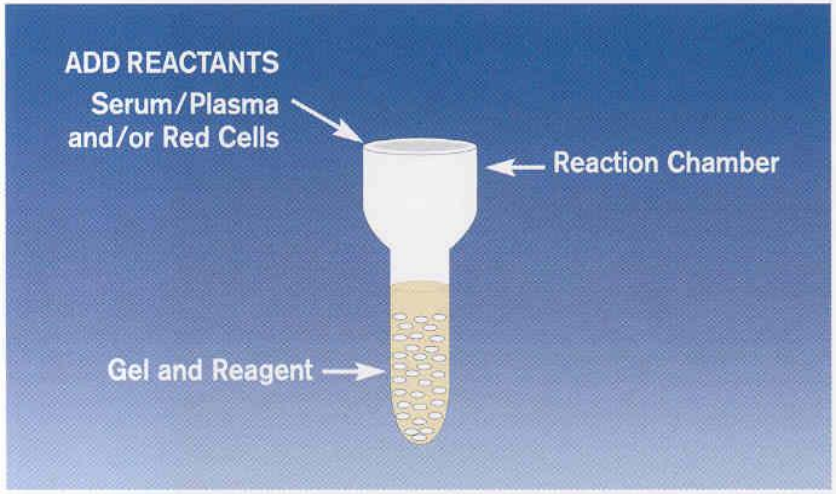
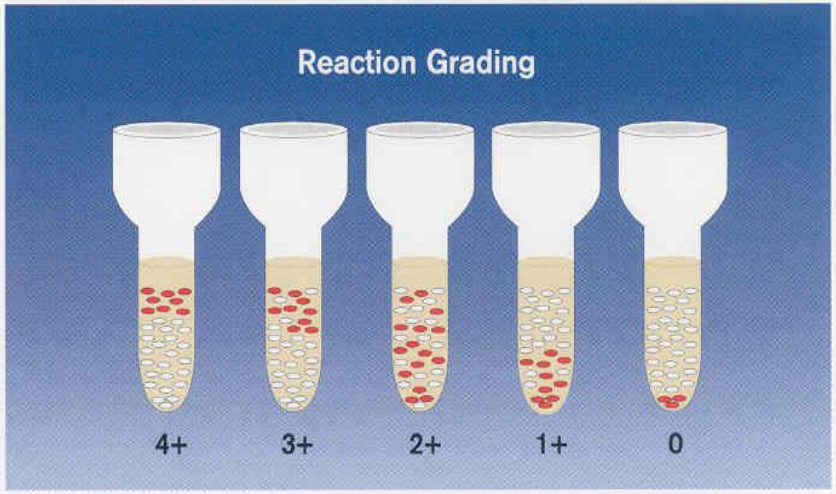


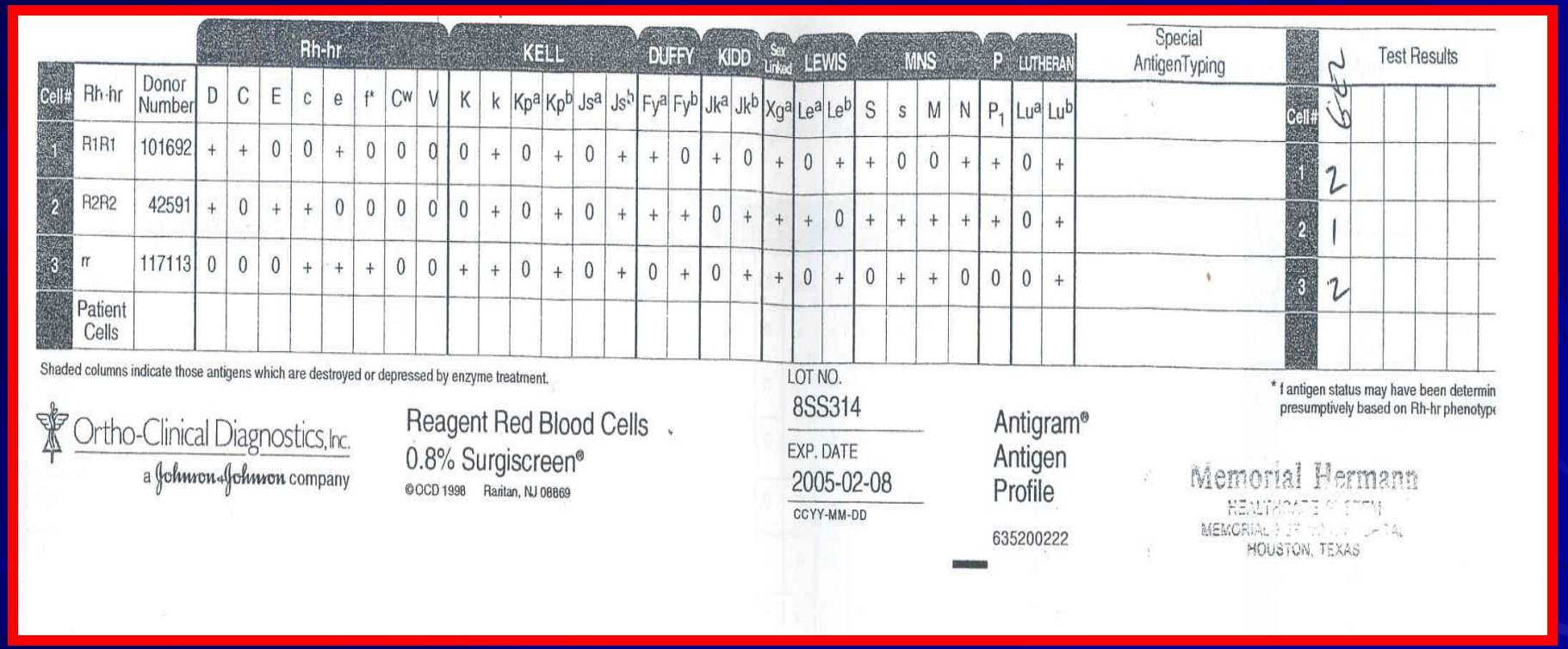
Illustration of a microtube.



The gel test yields reactions that are graded from 0 to 4+. This is similar to the grading used in traditional tube testing.

Ortho gel

Antibody Screen



			Rh-hr								KELL						DUFFY		KIDD		Sex Linked	LEWIS		MNS				P	LUTHERAN		Special AntigenTyping	Test Results
Cell #	Rh-hr	Donor Number	D	C	E	c	e	f*	Cw	V	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Xg^a	Le^a	Le^b	S	s	M	N	P_1	Lu^a	Lu^b		Cell #
1	R1R1	101692	+	+	0	0	+	0	0	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	0	0	+	+	0	+		1
2	R2R2	42591	+	0	+	+	0	0	0	0	0	+	0	+	0	+	+	+	0	+	+	+	0	+	+	+	+	+	0	+		2
3	rr	117113	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	0	+	+	0	+	0	+	+	0	0	0	+		3
	Patient Cells																															

Shaded columns indicate those antigens which are destroyed or depressed by enzyme treatment.

Ortho-Clinical Diagnostics, Inc.
a Johnson & Johnson company

Reagent Red Blood Cells
0.8% Surgiscreen®
©OCD 1998 Raritan, NJ 08869

LOT NO.
8SS314

EXP. DATE
2005-02-08
CCYY-MM-DD

Antigram®
Antigen
Profile

635200222

* f antigen status may have been determin
presumptively based on Rh-hr phenotype

Memorial Hermann
HEALTHCARE SYSTEM
MEMORIAL HERMANN HOSPITAL
HOUSTON, TEXAS

Screening Panel

Cell	AHG
SC I	neg
SC II	2+
Auto	neg

Possible Interpretation

- single alloantibody
- two antibodies, antigen present in cell II only
- probable IgG antibody

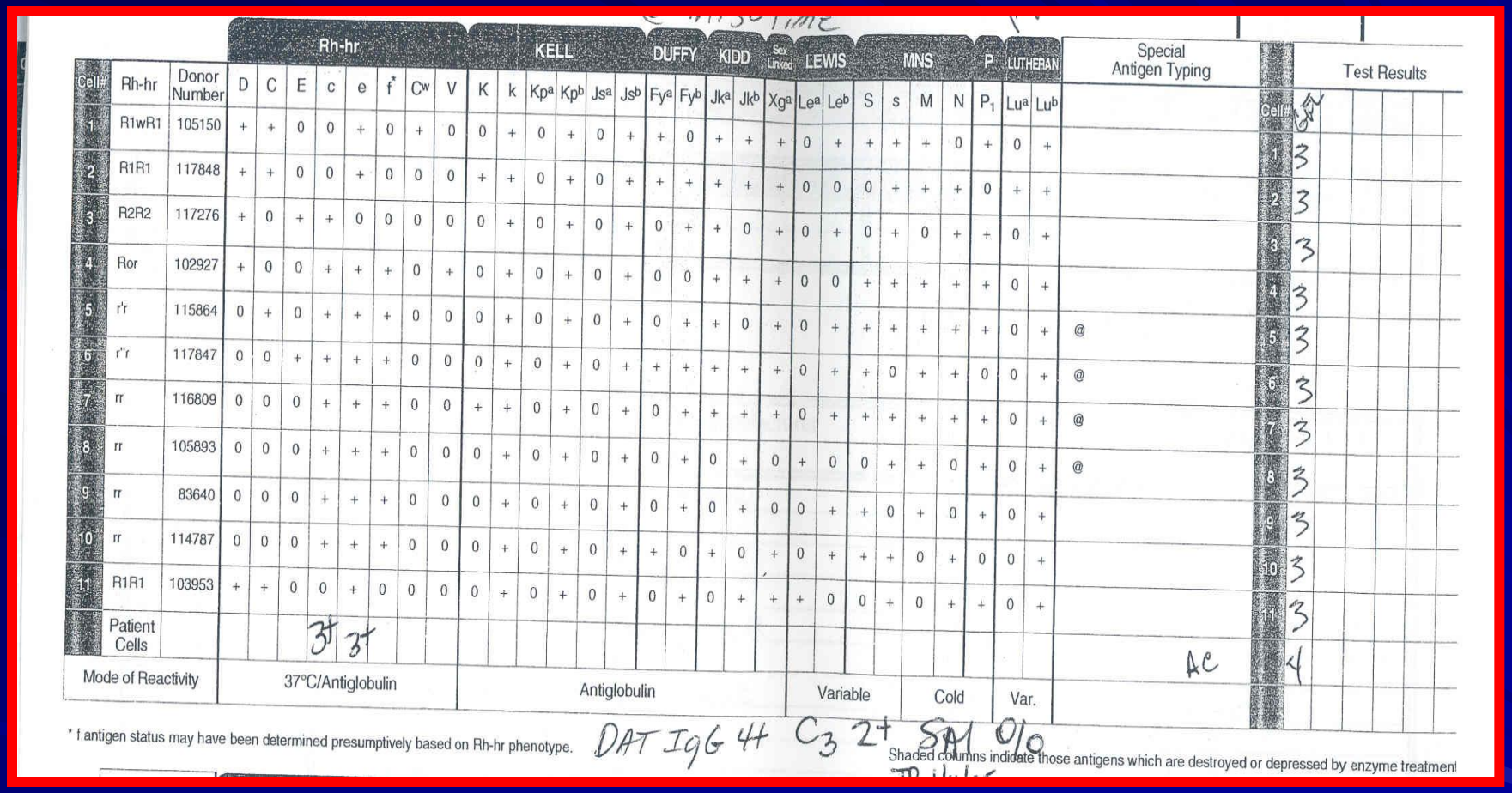
cell	AHG
SC I	3+
SC II	1+
Auto	neg

- multiple antibodies
- single antibody
- probable IgG

Antibody identification

- To determine specificity of antibody
- Tube, Gel, SPECA, methodologies
- Can use enhancement media like LISS, albumin, PEG, AHG, enzymes
- Honor previous antibody even if undetectable
  - 30-35% antibodies become undetectable in 1 year, and 50% are undetectable in 10 or more years
- Autocontrol or DAT not required

Antibody identification panel



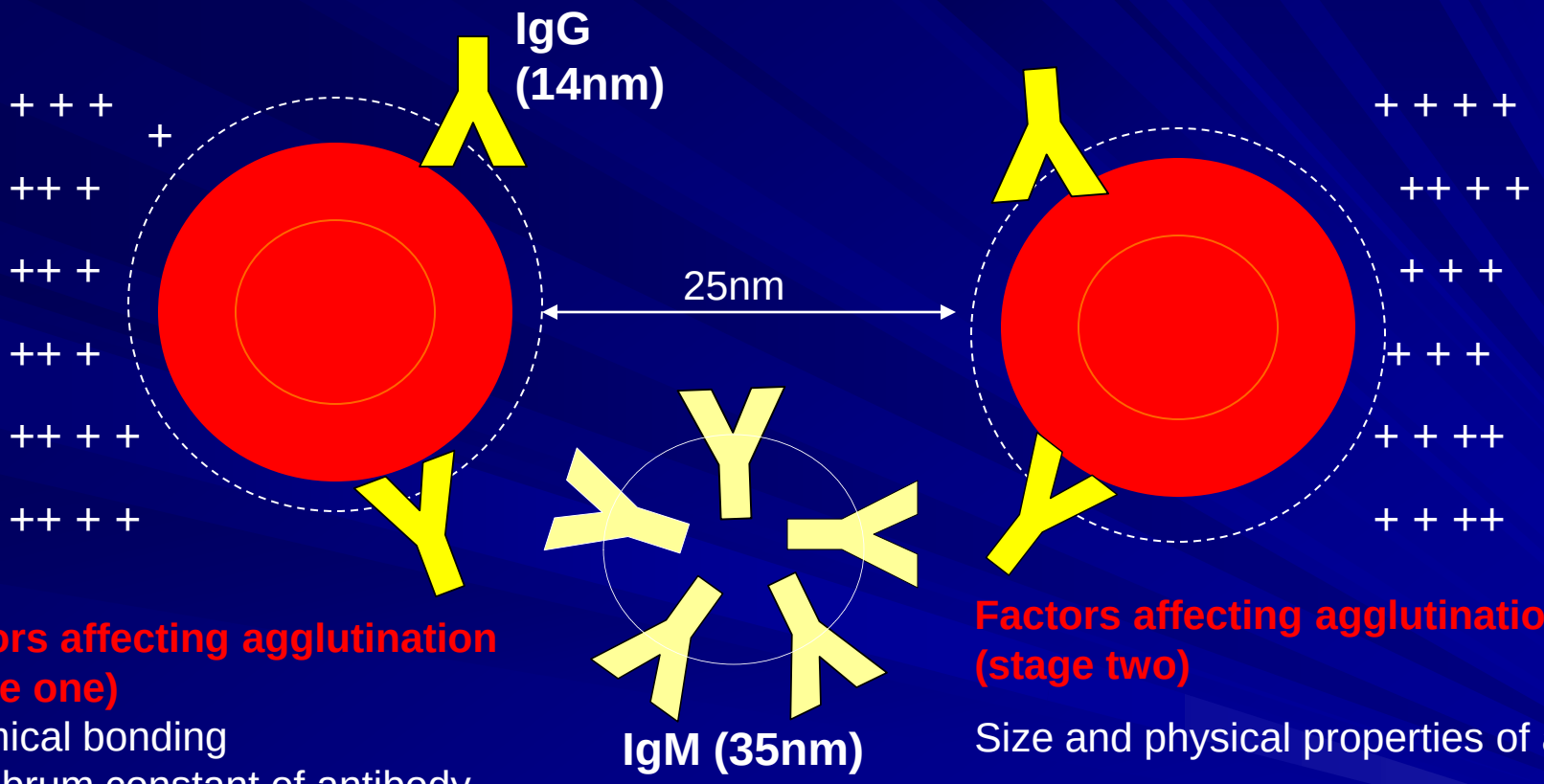
Cell#	Rh-hr	Donor Number	Rh-hr D	Rh-hr C	Rh-hr E	Rh-hr c	Rh-hr e	Rh-hr f*	Rh-hr Cw	Rh-hr V	KELL K	KELL k	KELL Kpa	KELL Kpb	KELL Jsa	KELL Jsb	DUFFY Fya	DUFFY Fyb	KIDD Jka	KIDD Jkb	Sex Linked Xga	LEWIS Lea	LEWIS Leb	MNS S	MNS s	MNS M	MNS N	P P1	LUTHERAN Lua	LUTHERAN Lub	Special Antigen Typing	Cell#	Test Results
1	R1wR1	105150	+	+	0	0	+	0	+	0	0	+	0	+	0	+	+	0	+	+	+	0	+	+	+	+	0	+	0	+		1	3
2	R1R1	117848	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	+	+	+	+	0	0	0	+	+	+	0	+	+		2	3
3	R2R2	117276	+	0	+	+	0	0	0	0	0	+	0	+	0	+	0	+	+	0	+	0	+	0	+	0	+	+	0	+		3	3
4	Ror	102927	+	0	0	+	+	+	0	+	0	+	0	+	0	+	0	0	+	+	+	0	0	+	+	+	+	+	0	+		4	3
5	r'r	115864	0	+	0	+	+	+	0	0	0	+	0	+	0	+	0	+	+	0	+	0	+	+	+	+	+	+	0	+	@	5	3
6	r''r	117847	0	0	+	+	+	+	0	0	0	+	0	+	0	+	+	+	+	+	+	0	+	+	0	+	+	0	0	+	@	6	3
7	rr	116809	0	0	0	+	+	+	0	0	+	+	0	+	0	+	0	+	+	+	+	0	+	+	+	+	+	+	0	+	@	7	3
8	rr	105893	0	0	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	+	0	0	+	+	0	+	0	+	@	8	3
9	rr	83640	0	0	0	+	+	+	0	0	0	+	0	+	0	+	0	+	0	+	0	0	+	+	0	+	0	+	0	+		9	3
10	rr	114787	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	0	+	0	+	0	+	+	+	0	+	0	0	+		10	3
11	R1R1	103953	+	+	0	0	+	0	0	0	0	+	0	+	0	+	0	+	0	+	+	+	0	0	+	0	+	+	0	+		11	3
	Patient Cells					3+	3+																							AC		4
Mode of Reactivity			37°C/Antiglobulin								Antiglobulin											Variable		Cold			Var.					

* f antigen status may have been determined presumptively based on Rh-hr phenotype. DAT IgG 4+ C3 2+ SAL 0/0

Shaded columns indicate those antigens which are destroyed or depressed by enzyme treatment

patient's serum is tested against a panel of blood group O rbcs

Zeta Potential



Factors affecting agglutination (stage one)

Chemical bonding
Equilibrum constant of antibody
Temperature
pH
Incubation time
Ionic strength
Antigen- antibody proportion

Factors affecting agglutination (stage two)

Size and physical properties of antibody

Concentration of antigen sites

Distance between cells

- *Zeta potential*
- *Water of hydration*
- *Van der Waals forces*

Enhancement media

Reagent	Action	Antibody
22% albumin	Adjusts zeta potential	IgG, may enhance cold abs
LISS	Increases rate of ab binding to RBC antigens	IgG, may enhance cold abs
Polybrene	Neutralization of charge	IgG, may be less sensitive for Kell
enzymes	Enhances some RBC antigens, depresses others Lowers zeta potential Interfacial tension Spicule formation	Enhances Rh, Kidd, $P_{1,}$ I, Lewis Destroys Duffy, MNS
Polyethylene Glycol	Removes water, promotes ab uptake and reaction strength	IgG, ↓IgM reactivity, false positives, enhances warm auto
AHG	Crosslinks sensitized cells → visible agglutination	Polyspecific or monospecific IgG

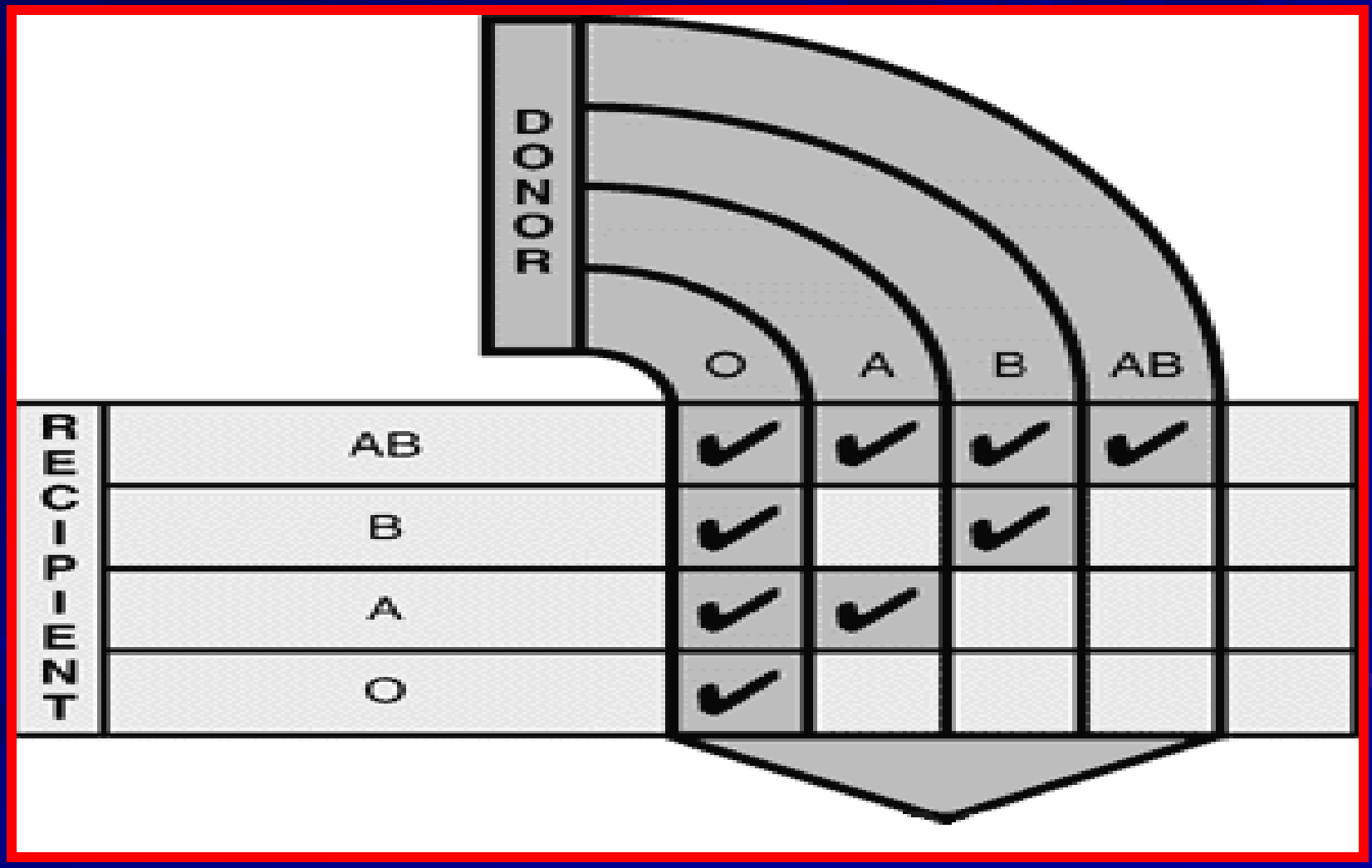
Crossmatch

- Final check of ABO compatibility
- May detect antibody not found on screening

Crossmatch

- Serologic
  - Immediate spin only if antibody screen is negative (abbreviated crossmatch)
  - Antiglobulin if antibody screen is positive
- Electronic or Computer crossmatch
  - if antibody screen is negative

Compatible RBC units



Frequency of + crossmatch and - screen

- Mintz study
  - 0.2% patients had positive crossmatches after a negative antibody screen
- Heddle study
  - 0.3 % patients had incompatible transfusions, no adverse outcome
- Havemann study
  - Calculated incidence 0.008%

Causes of Incompatible crossmatches

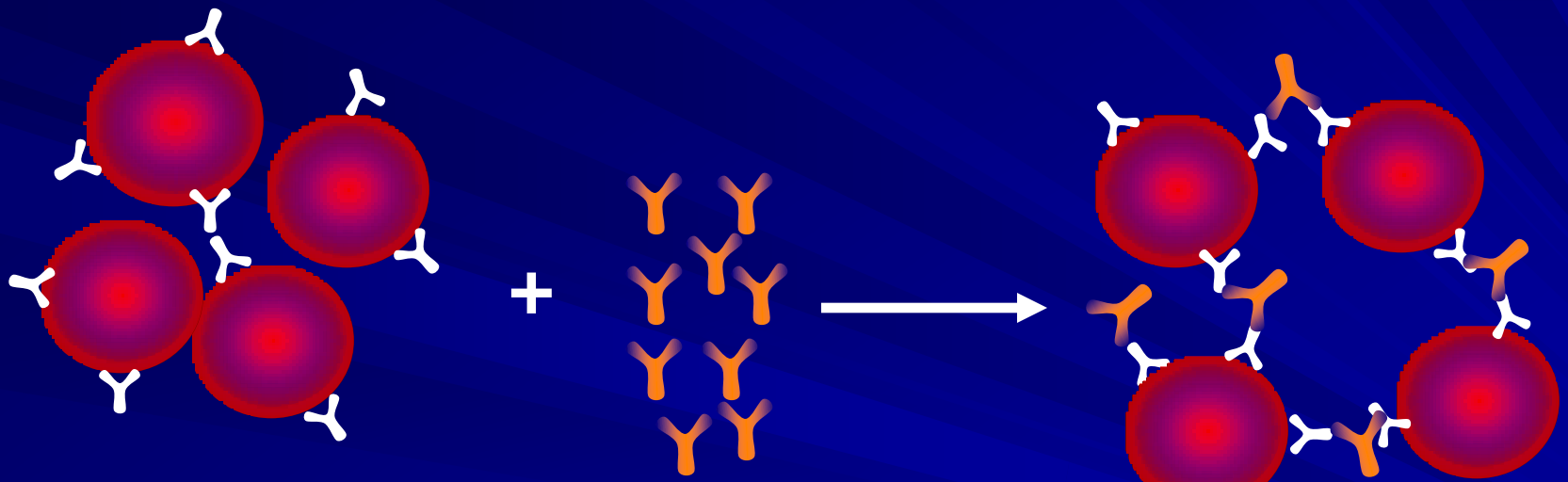
- incorrect ABO grouping
- alloantibody in patient serum
- autoantibodies
- positive DAT on donor cells
- abnormalities in patient serum
  - imbalance of albumin/ gamma globulin ratio
  - plasma expanders
  - Antibody to additives
- Contaminants in test system

Direct Antiglobulin testing

- tests for sensitization of red cells by antibody or complement

  Autoimmune hemolytic anemia
  Hemolytic disease of the newborn
  Hemolytic transfusion reactions
  Drug induced
  idiopathic

Direct Antiglobulin test



Patient red blood cells

Anti- IgG (AHG or Coombs serum)

agglutination

Turn around time

‘But I sent in the sample 10 minutes ago!’

Turn - Around time

- RBC
  - emergency - immediately
  - emergency incomplete - 15mins
  - emergency complete - 60 mins
  - elective complete - 2 - 4 hours

- FFP, cryoprecipitate – 30- 60mins
- platelets - 30mins (for pooled)

Emergency Transfusion

Especially seen in trauma and surgical cases

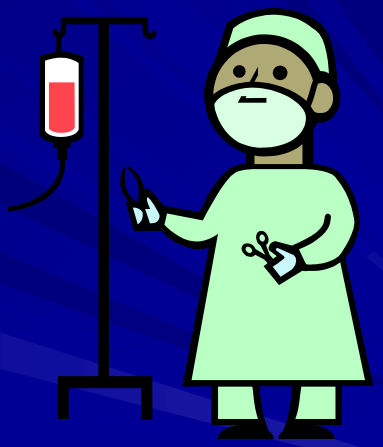
May need to transfuse blood before testing is completed

Emergency Transfusion

- Physician will need to sign an emergency release form, waiving compatibility testing
- The blood bank will immediately issue
  - Uncrossmatched O negative RBCs
    - We need a patient sample ASAP

OR

  - Uncrossmatched group specific RBCs
    - If you can wait 5- 10 minutes
    - If patient's type is already known



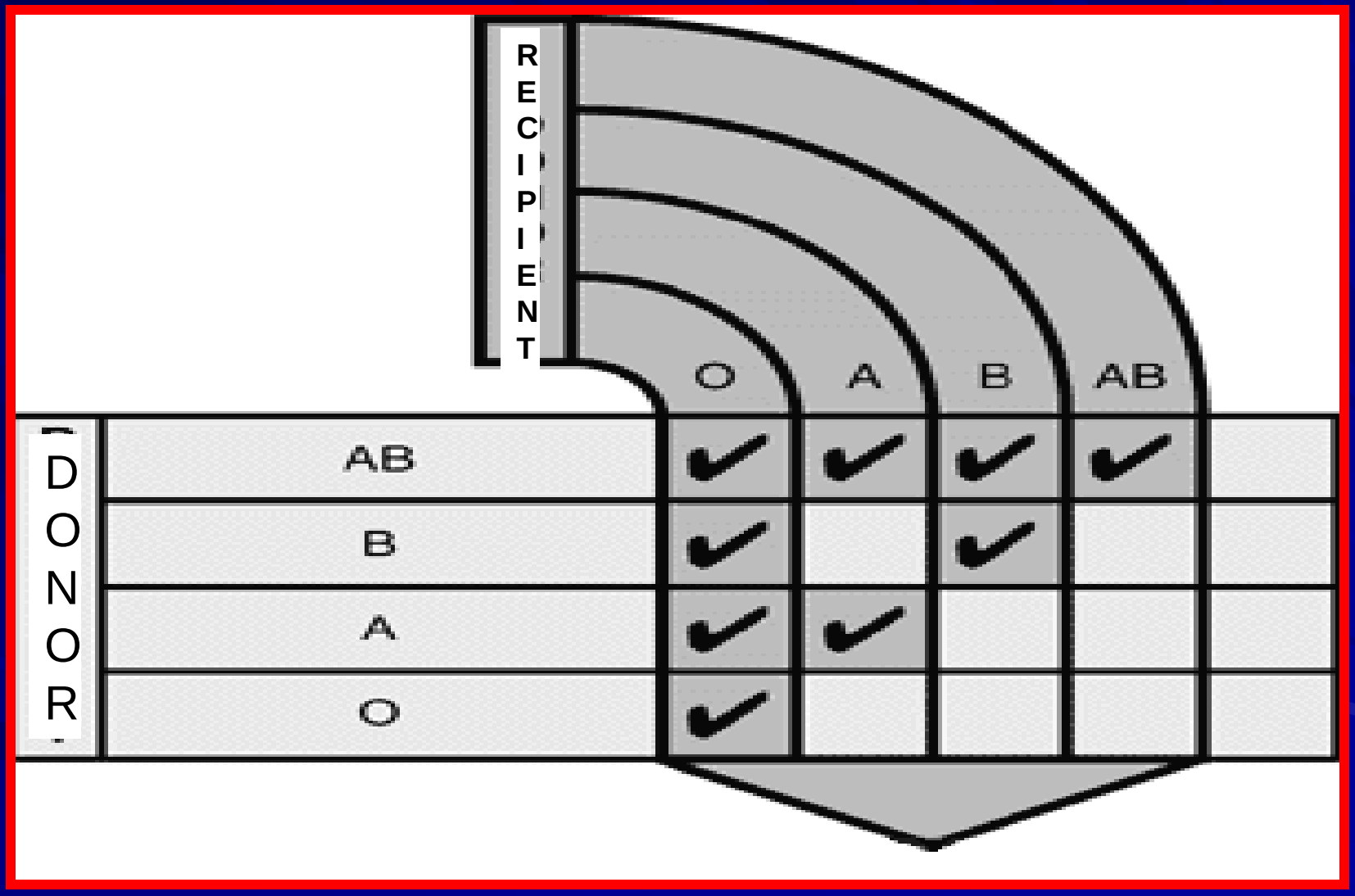
Other Serologic Techniques

- Enzyme treatment
- Elution
- Adsorbtion
- Neutralization
- Chloroquine diphosphate
- Quantification of antibody

Plasma Products

- Test for ABO group
- no crossmatch required
- plasma or plasma product should be compatible with recipient ABO blood group.

Compatible Plasma



Compatibility testing for infants (less than 4 months old)

- ABO, Rh testing
- Alloimmunization to RBC antigens is rare
- Antibody screen
  - using maternal serum or plasma
  - using infants serum or plasma
  - infants eluate

Compatibility testing for infants (less than 4 months old

- Repeat compatibility testing may be omitted during one hospitalization

As long as several criteria are met

Initial screen is negative

Transfused cells are group O or ABO identical

Rh negative or Rh identical

Crossmatch/ Transfusion ratio

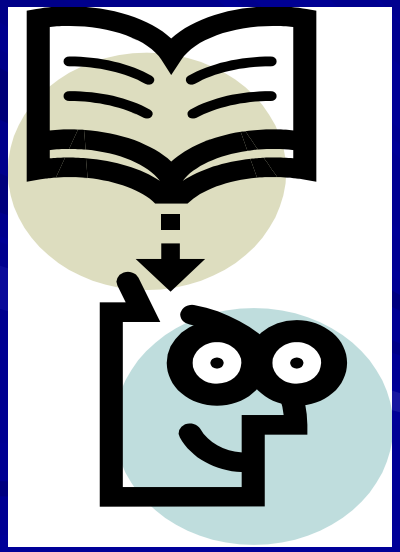
- Ratio of crossmatched units to actually transfused units for a patient
- C:T >2.) indicates excessive crossmatch requests
- Ordering guidelines available for different surgical procedures

Other Terminology

- Maximum surgical blood order schedules (MSBOS)
  - Use data to determine if a T/S order or a certain number of units is required for different elective surgical procedures
- Standard blood order system(SBO) – modification of MSBOS in some institutions

Problems you will encounter

- Multiple antibodies
- Antibodies to high frequency antigens
- Antibodies to low frequency antigens
- Cold or warm autos
- Antibodies to reagents
- Incompatible crossmatch
- Emergency release
- Switch Rh type



Dr Smart, Path resident

How do I determine how many units to screen when a clinically significant antibody is detected?

- Need to know incidence of antigens

Example: Pt has anti K and needs 4 units RBC

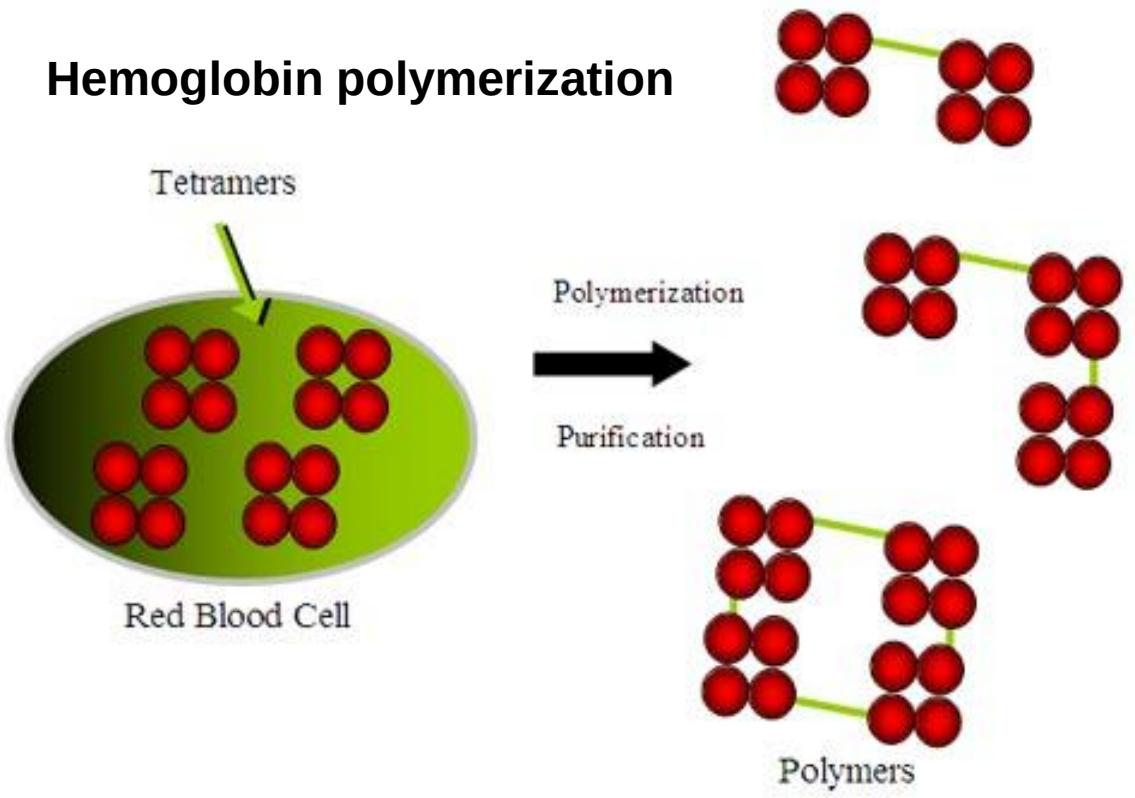
K - incidence is 90% = 0.90

$$\frac{\text{4 units K- blood needed}}{0.90} = 4.4 \text{ units}$$

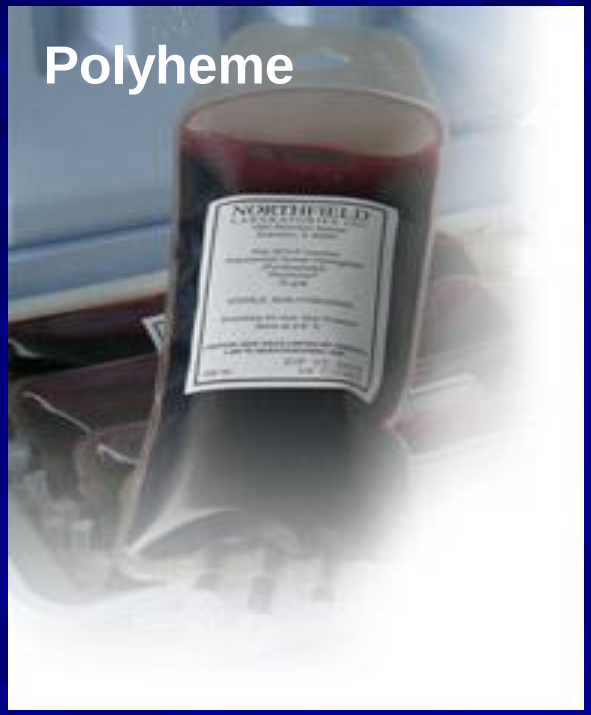
Future of Compatibility testing

- RBC substitutes
- biochemical modification of all non O blood groups
- automation
  - solid phase
  - galvanic testing
  - gel testing
- dipstick tests
- dry plate testing

Hemoglobin polymerization

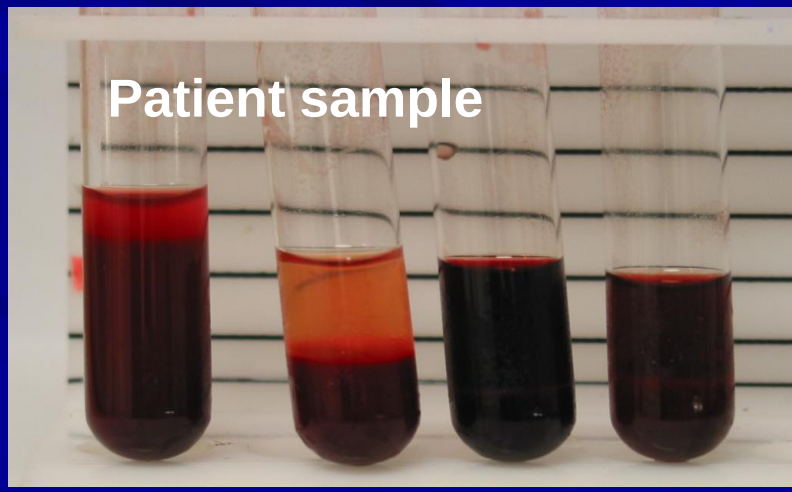


Polyheme

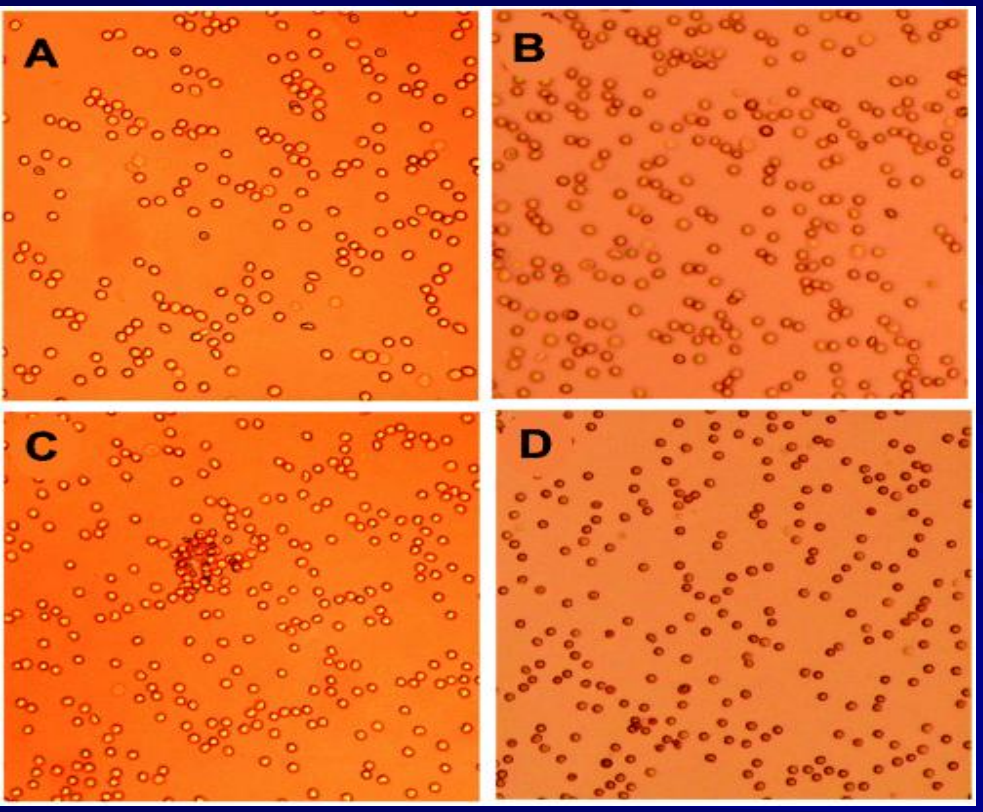


http://www.northfieldlabs.com/polyheme

Patient sample



Biochemical modification of all non O blood groups



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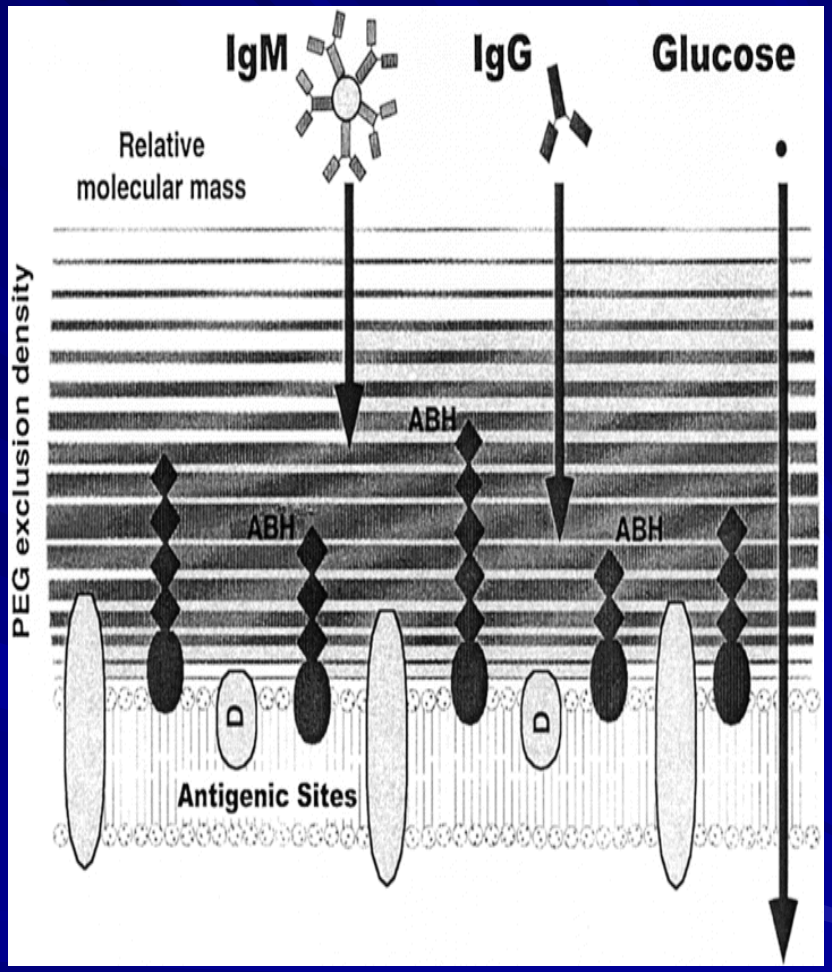
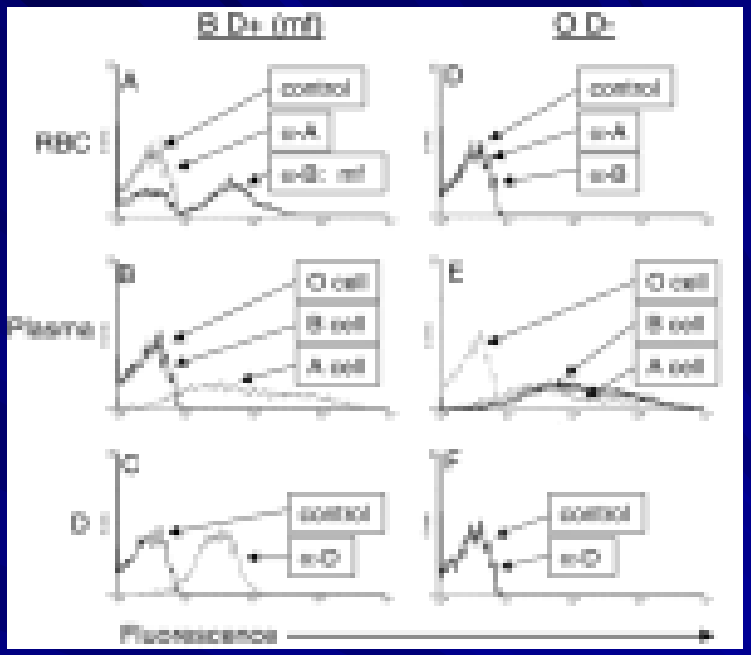
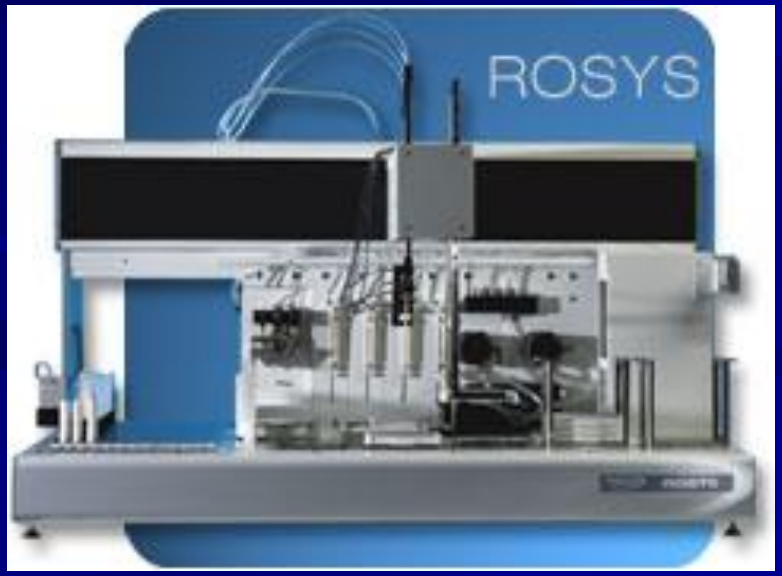


Transfusion
Volume 41 Issue 10 Page 1225 - October 2001

Automation

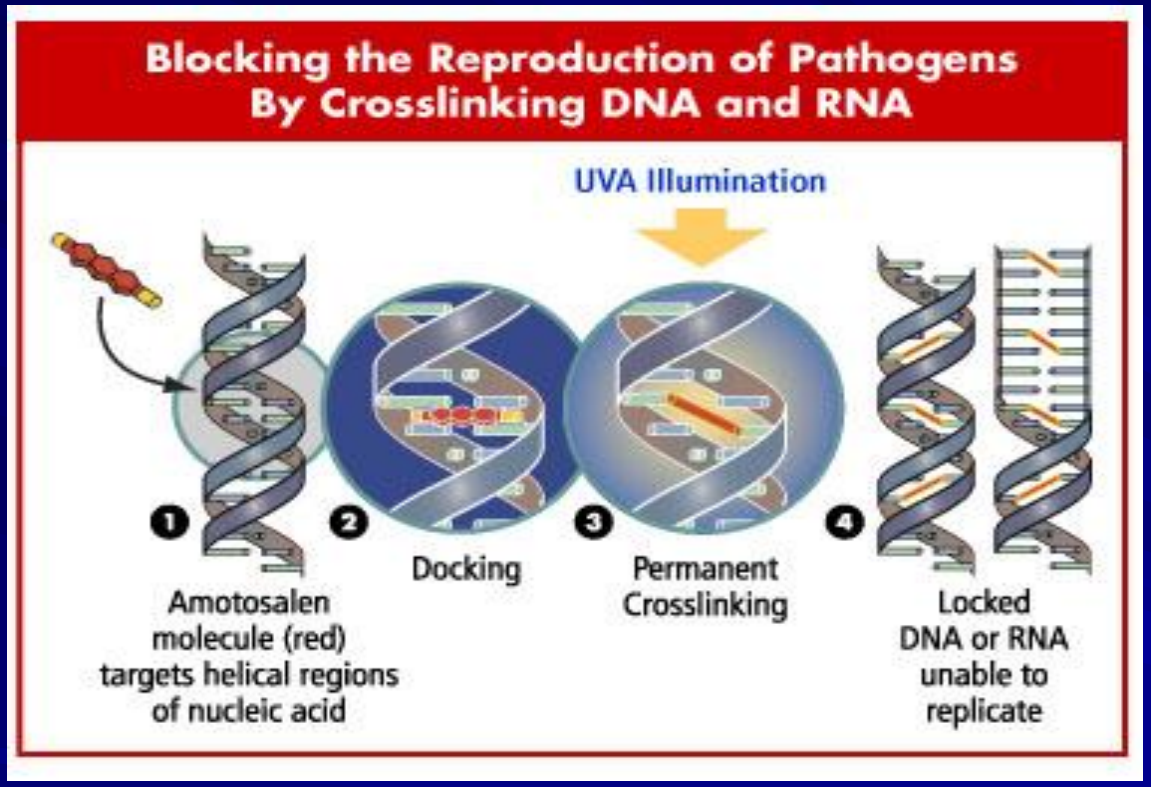

Galileo

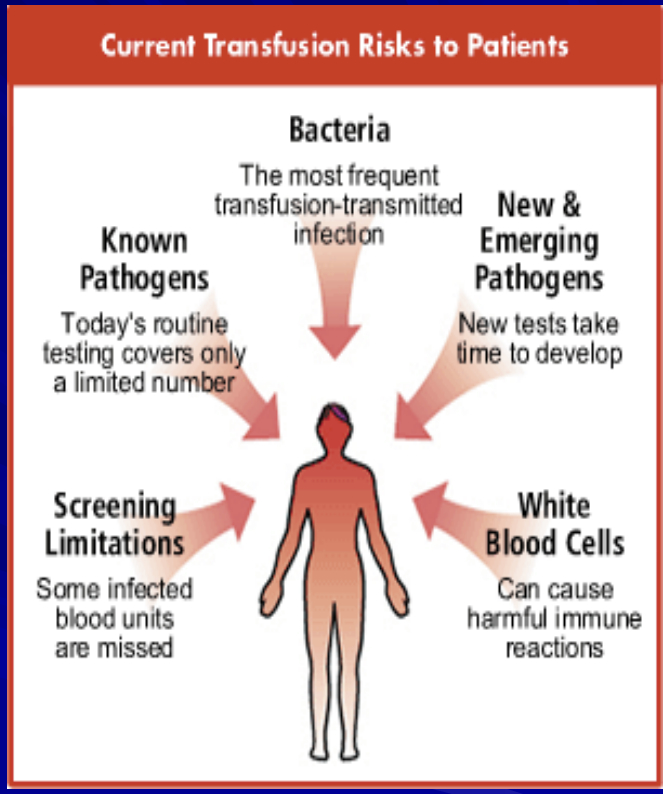

B D+ (mf)
O D-
RBC
control
anti-A
anti-B
Plasma
O cell
B cell
A cell
Fluorescence


ROSYS


VUE

Pathogen inactivation

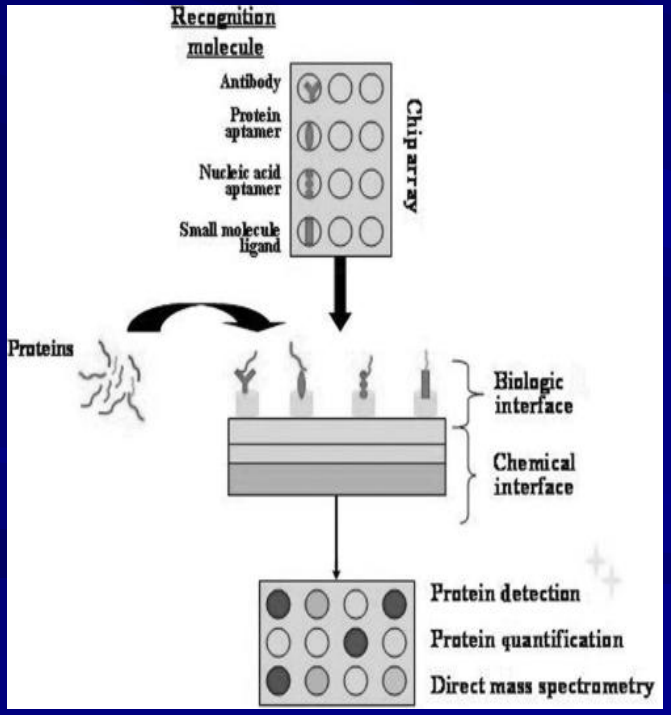


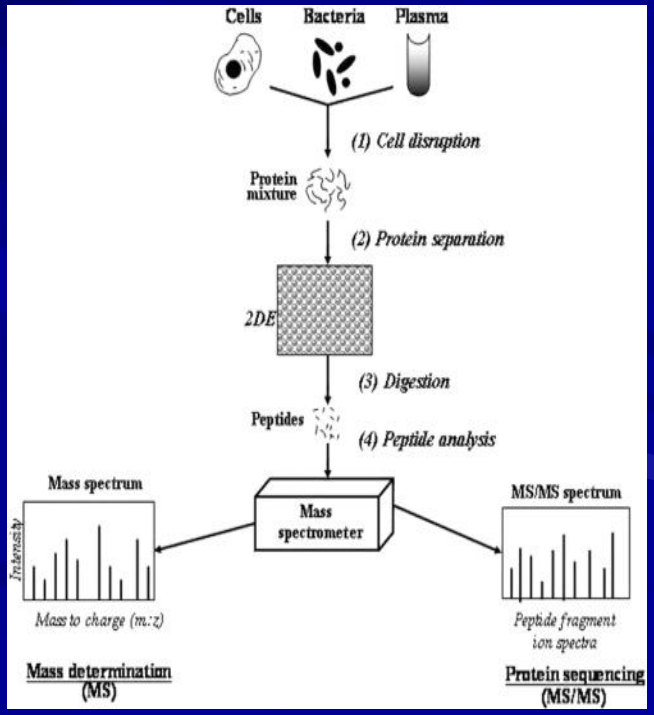


http://www.interceptbloodsystem.com

Future contd

- Genomic microarray Technology and Proteomic analysis
- Blood molecular genotyping





Transfusion Volume 44 Issue 4 Page 601 - April 2004

"Personally, I wouldn't have signed it."

http://www.jwolfe.clara.net

Quiz

- How did protein S come about its name?

CLUE: a city

Protein S is named S for Seattle, because the first patient described with protein S deficiency was in this city.

http://www.stago.com/gb/asp/home_global.asp