Antibody Screening (RAI)

S.Pouya- 13 february 2018

Ortho Clinical Diagnostics

Welcome

Blood Group Antigens

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Blood Group: Antigens

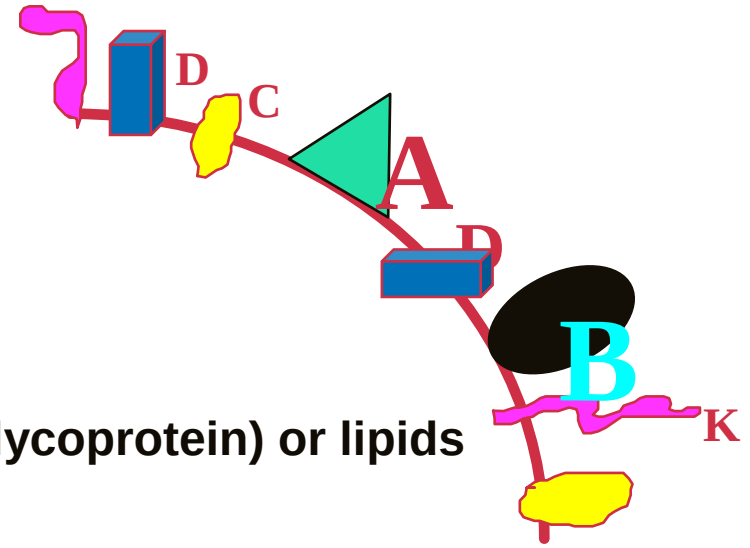
- **Blood groups are represented by molecules: Antigens.**
- **Antigens are able to induce an immune response.**
- **Different type of antigens exist:**
  - Hetero-antigens: from another species
  - Allo-antigens: from the same species
  - Auto-antigens: from the same individual
- **More than 300 blood group antigens discovered so far classify within 35 systems**

Blood Group: Antigens

- **System ABO:** antigen A, B…
- **System Rhesus**: antigen D,C,E,e,c,Cw,…
- **System Kell:** antigens K, k, Kpa, Kpb, …
- **System Lutheran**: Lua, Lub, …
- **System Lewis**: antigen Lea, Leb,…
- **System MNS**: antigens M, N, S, s, …
- ….35 systems >300 antigens

Blood Group: Antigens

- **Antigens are present on the surface of red blood cells**



- **Chemical structures made of:**
  - **Protein**
  - **Carbohydrate determinants on proteins (glycoprotein) or lipids (glycolipid)**

- **Function: membrane structures, membrane transport, receptors...**

Antigens give the identity of each individual

Blood Group Antibodies

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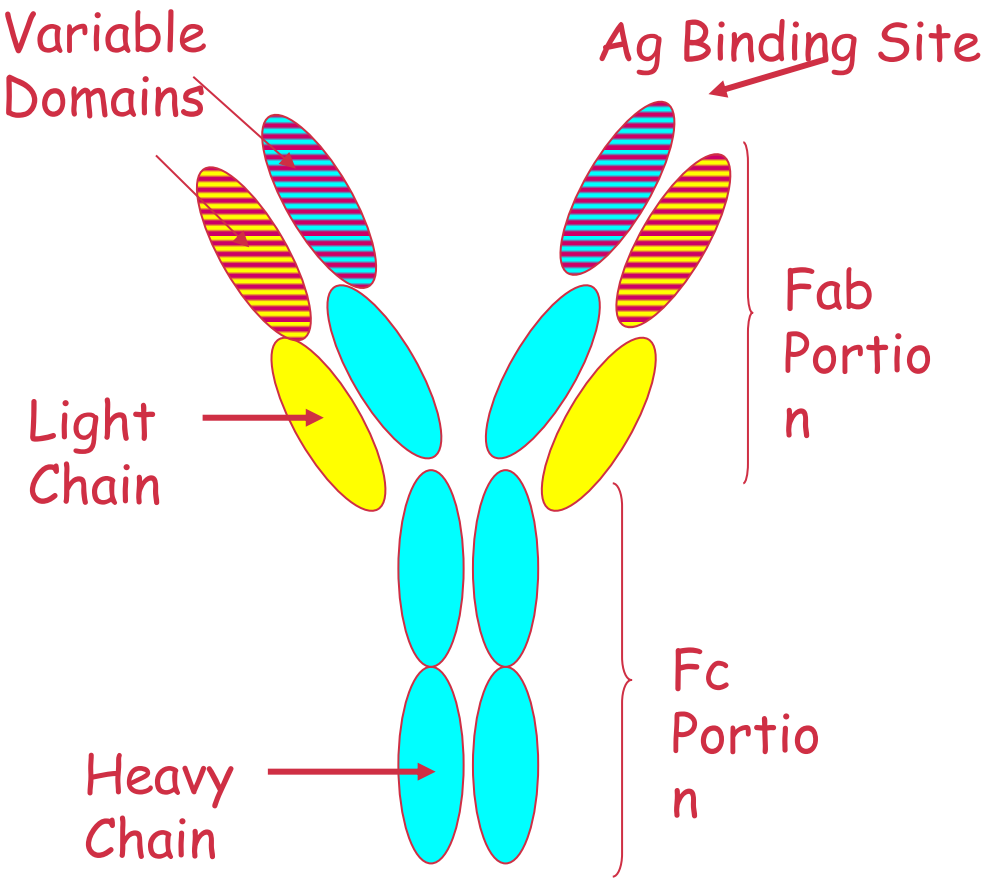
Immune Response

- Reaction to the invader (Antigen)
- The ability of an antigen to stimulate the production of its corresponding antibody is called **immunogenicity**
- An Antibody (Ab) is a chemically complex protein molecule produced in response to an antigen
- Immunization to red cell antigens may result from pregnancy, transfusion, transplant

Antibodies (Ab)

- Serum/plasma contain antibodies
- Antibodies are molecules that can destroy red blood cells
- Antibodies can be dangerous in Blood transfusion and must be *screened*

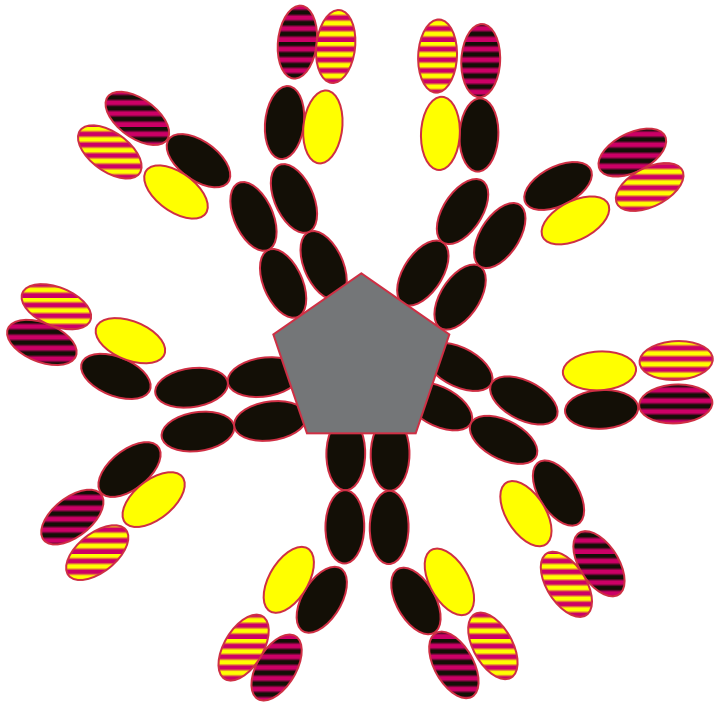


Immunoglobulin (Antibody)

Structure

IgG

IgA, IgD, IgE

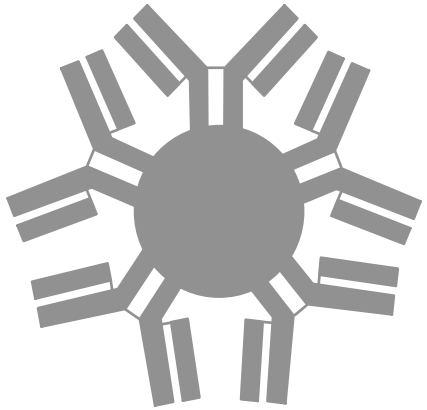


IgM

Blood Group Antibody

Natural antibodies:

"naturally occurring" Individuals can make Ab to antigens (Ag) NOT present on their own red cells - alloantibodies e.g. anti-A and anti-B



IgM

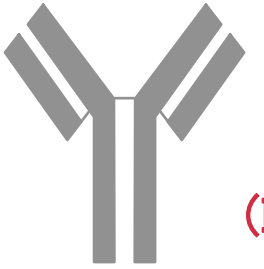
Immune antibodies:

Unexpected antibodies produced after exposure to foreign red cell antigens

Red Cell Transfusion

Pregnancy

Transplant …

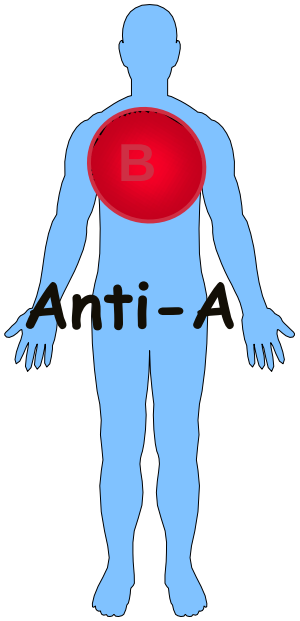


IgG

(IgA, IgD, IgE)

Blood Group Antibody

"Naturally" occurring

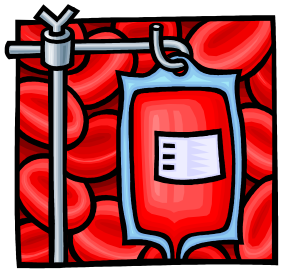


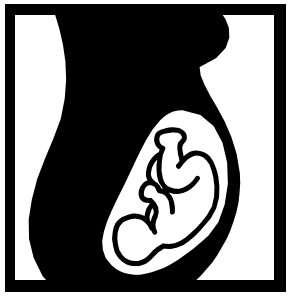
Typically:

- **IgM**
- **Directly** agglutinate RBC
- Optimally react at **<30ºC** (cold antibodies)
- **Fix the complement**
- Elicited by carbohydrate antigens

Examples:
Expected: Anti-A &/or Anti-B
Unexpected: Anti-Le^a, Anti-M, Anti-P_1

Immune stimulated





Typically:

- **IgG**
- **Indirectly** agglutinate RBC
- Optimally react at **37ºC** (warm antibodies)
- Most **not** able to **fix** the complement
- **Cross placenta**
- Elicited by protein antigens

Examples: **all unexpected**
Anti-D, Anti-C, Anti-K
Anti-Jk^a, Anti-Fy^a, Anti-Fy^b

Antibody Screening

Test used to detect/ confirm presence/absence of unexpected blood group antibodies in serum/plasma

Ensure safe transfusion

Detect/monitor HDN

The Basics…

– When detecting and/or identifying antibodies, we test

Known:		Unknown:
Reagent RBCs	**+**	**patient serum**

– Antibody Screens use 2 or 3 Screening Cells to “detect” if antibodies are present in the serum/plasma

– If antibodies are detected, they must be identified

Identification test

- **Screening Cells and Panel Cells are the same with minor differences:**
  - Screening cells
    - Antibody detection
    - Sets of 2 or 3 vials
  - Panel cells
    - Antibody identification
    - At least 10 vials per set

An antibody panel is just an extended version of an antibody screen

Reagents

Group O red cells for antibody screening of serum or plasma are commercially available and are offered as sets of either two or three vials of single-donor red cells.

Reagent cells must express the following antigens: D, C, E, c, e, M, N, S, s, P1, Lea , Leb , K, k, Fya , Fyb , Jka and Jkb

>> for additional antigens, refer to local regulations

Reagents

in France, the screening panel needs to contain:

- At least 3 test RBC group O
- On this panel following antigens must be present: D, C, E, c, e, K, k(cellano), Kpb, Fya, Fyb, Jka, Jkb, M, N, S, s, Lea, Leb, P1, Lub
- The following phenotypes must be present :Rh: 1,2,-3,-4,5; Rh: 1,-2, 3,4,-5; Rh: -1,-2,-3,4,5.
- Antigens Fya, Jka, Jkb, S must be homozygous.

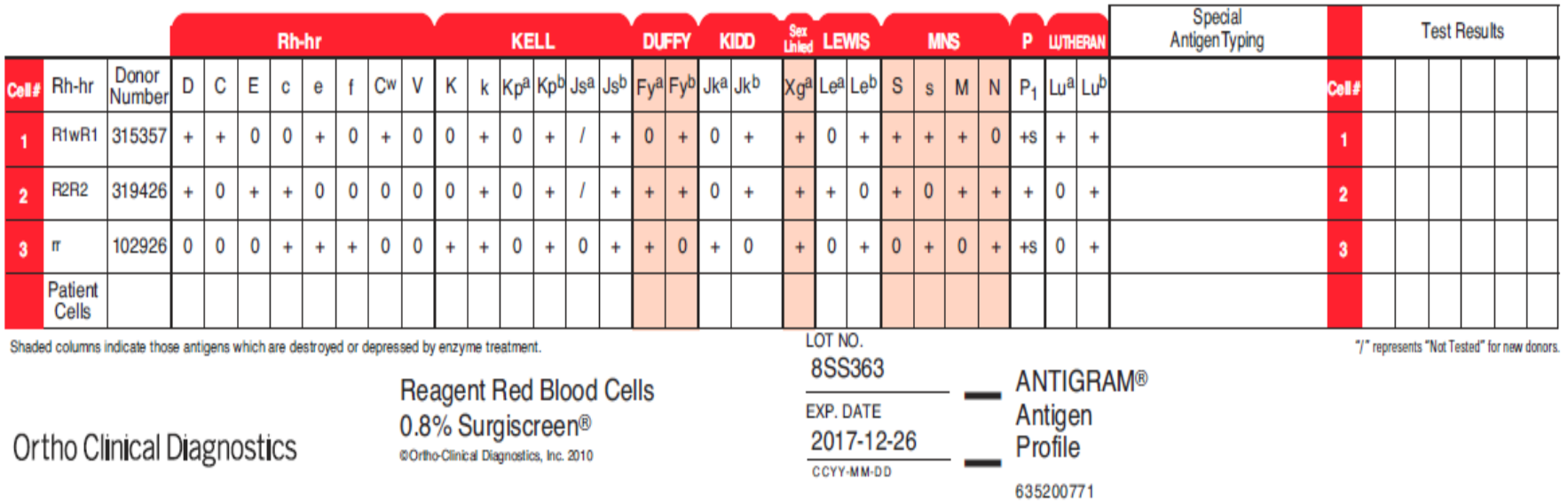
Recommended homozygous antigens for Fyb and s

Panel

Each of the panel cells has been antigen typed (shown on antigram)

+ refers to the presence of the antigen

0 refers to the absence of the antigen



			Rh-hr								KELL						DUFFY		KIDD		Sex Linked	LEWIS		MNS				P	LUTHERAN		Special Antigen Typing		Test Results						
Cell #	Rh-hr	Donor Number	D	C	E	c	e	f	C^w	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	R1wR1	315357	+	+	0	0	+	0	+	0	0	+	0	+	/	+	0	+	0	+	+	0	+	+	+	+	0	+s	+	+		1						
2	R2R2	319426	+	0	+	+	0	0	0	0	0	+	0	+	/	+	+	+	0	+	+	+	0	+	0	+	+	+	0	+		2						
3	rr	102926	0	0	0	+	+	+	0	0	+	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	+	+s	0	+		3						
	Patient Cells																																				

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

"/" represents "Not Tested" for new donors.

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Reagent Red Blood Cells
0.8% Surgiscreen®
©Ortho-Clinical Diagnostics, Inc. 2010

LOT NO.
8SS363
EXP. DATE
2017-12-26
CCYY-MM-DD

ANTIGRAM®
Antigen
Profile
635200771

			Rh-hr								KELL						DUFFY		KIDD		Sex Linked	LEWIS		MNS				P	LUTHERAN	
Cell #	Rh-hr	Donor Number	D	C	E	c	e	f	C^w	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
1	R1wR1	315357	+	+	0	0	+	0	+	0	0	+	0	+	/	+	0	+	0	+	+	0	+	+	+	+	0	+s	+	+
2	R2R2	319426	+	0	+	+	0	0	0	0	0	+	0	+	/	+	+	+	0	+	+	+	0	+	0	+	+	+	0	+
3	rr	102926	0	0	0	+	+	+	0	0	+	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	+	+s	0	+
	Patient Cells																													

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

LOT NO.
8SS363

EXP. DATE
2017-12-26
CCYY-MM-DD

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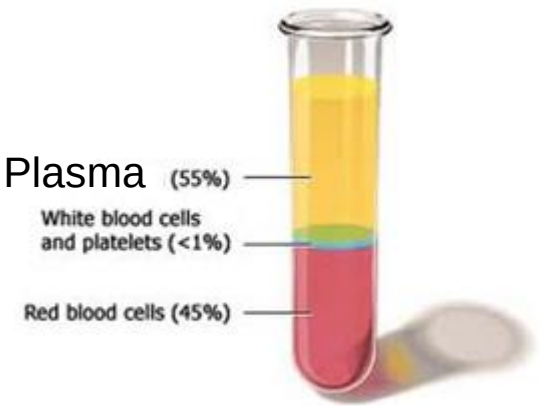
Reagent Red Blood Cells
0.8% Surgiscreen®

ANTIGRAM
Antigen
Profile

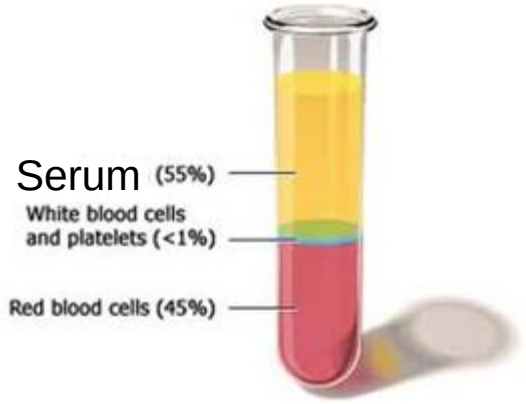
635200771

Blood specimens

To be use with the BioVue method, sample tubes need to be centrifuge 5 minutes between 900 and 1000 G



Blood taken on tube with anticoagulant



Blood taken on tube without anticoagulant

Unexpected Antibodies

Complement

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Complement & Hemolysis

- **The Complement System is a group of 18 to 20 naturally occurring soluble proteins dissolved in the blood plasma that provide immune protection.**
- **The function of the complement system is to generate activated complement proteins that lead to antigen destruction.**
- **Complement proteins become activated in a chain reaction sequence.**

Complement & Hemolysis

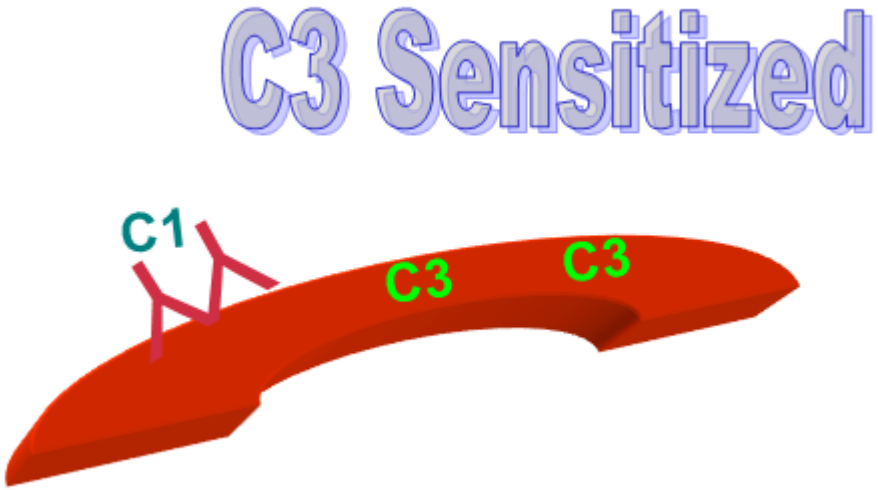
- **IgM or IgG binds to Ag (RBC)***
- **1st component of complement (C1q) binds to Fc portion of IgM or adjacent IgG antibodies**

- **Initiates a “cascade” or series of activations of complement components, some bind to the RBC**

* Aggregates of immunoglobulins can also initiate complement activation via an alternate pathway

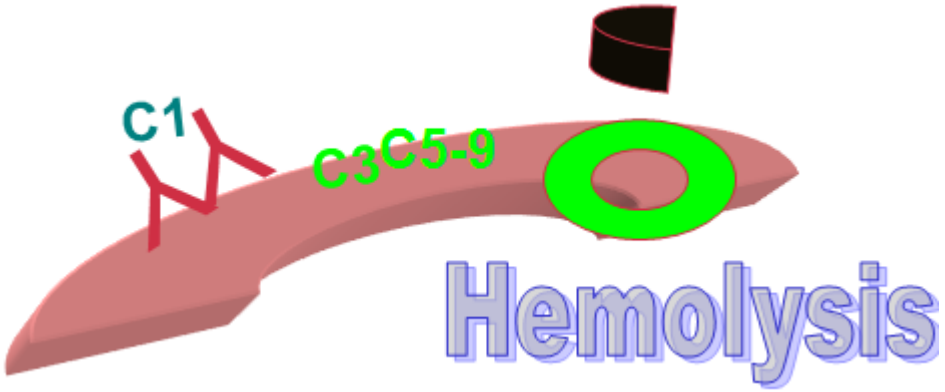
Complement & Hemolysis

- **C3 Activation**
  - **C3b binds to the RBC membrane; C3a fluid phase**
  - **C3b sensitized (coated) RBC can adhere to cells that have C3b receptors (phagocytes)**
  - **C3b is rapidly cleaved *in vivo* leaving C3d on the RBC surface.**
  - **Presence of C3d on RBC indicator of *in vivo* complement activation**



Complement & Hemolysis

When all components of complement are activated (C1 –> C9) the RBC membrane is damaged & lysis occurs



The complement activation needs the presence of Ca++ and Mg++

Unexpected Antibodies

Agglutination

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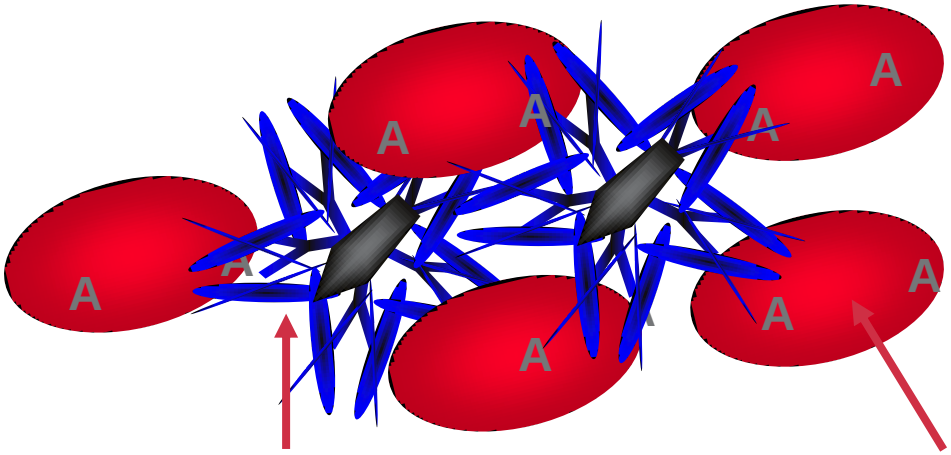
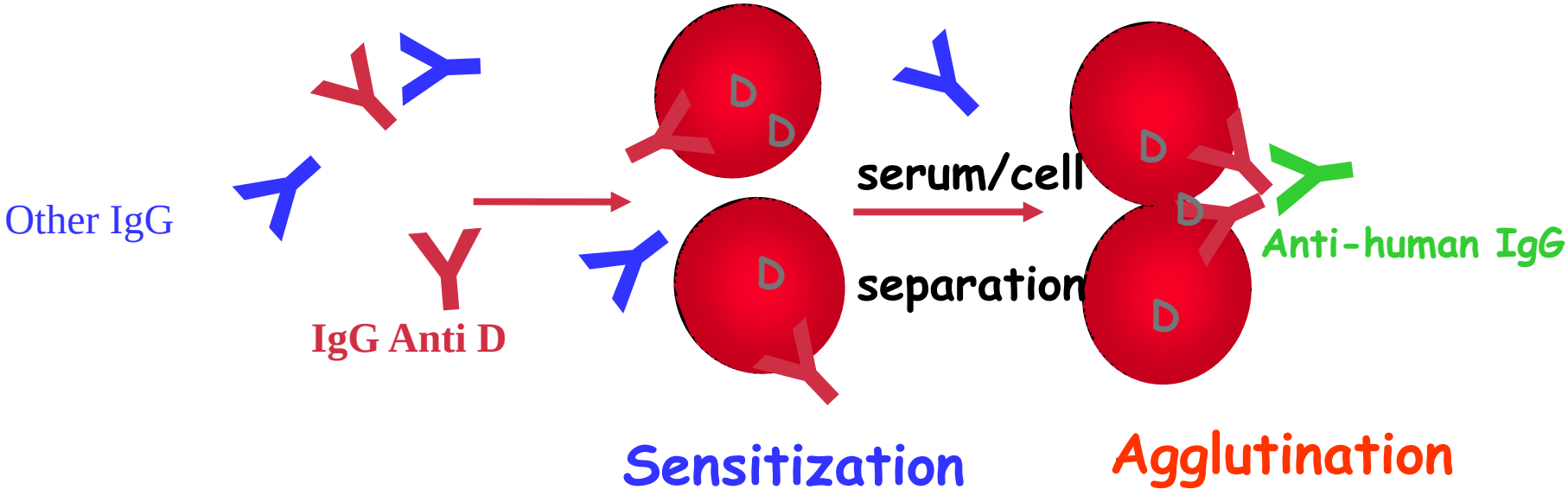
Direct Agglutination



- **Reaction is Visible = Agglutination**
- **In solution RBC are negatively charged and repel each other**
- **IgM antibody large enough to reach antigens on negatively charged RBC**

Indirect Agglutination *



- **Agglutination occurs using a second antibody**
- **IgG antibodies cannot "bridge" the gap between RBC**
- **IgG antibodies bind (sensitize), but do not agglutinate RBC**

*Basis of Indirect Anti-human globulin Test (IAT) or AHG Test

In Vitro RBC Ag/Ab Reactions

- **Antibody binds to antigen on RBC surface**

- **Binding detected by Agglutination, Hemolysis**
- **Conditions that affect [Ag_Ab] reaction**
  - Ab class (IgM, IgG) and concentration
  - Antigen :
    - Accessibility close to cell surface
    - Density
  - Environment: pH, ionic strength, temperature, time
  - Affinity of antibody for antigen

Unexpected Antibodies

Anti-Human Globulin

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Anti-Human Globulin (AHG)

Major Plasma proteins: **Fibrinogen**

Albumin

Globulins

They are separated by electrophoresis

In the globulins family we have:

Gamma globulins = immunoglobulin (IgM, IgG, IgA, IgD, IgE)

Beta globulins = complement components

Anti-Human Globulin (AHG)

Rabbits injected with human globulins produce anti-human globulin (AHG)*

- Anti-human gamma globulin: i.e. Anti-human IgG
- Anti-human beta globulin: i.e. Anti-human complement

* More specific antibodies can be made by using purified antigens (IgG or IgG heavy chain) or by producing monoclonal antibodies

Anti-Human Globulin (AHG)

Anti-Human Globulin can be

- ***Polyspecific*:**

Anti-IgG and anti-C3b-C3d are pooled into one reagent antiserum.

- ***Monospecific:***

Anti-IgG or anti-C3b-C3d are the component of the reagents

Test Theory

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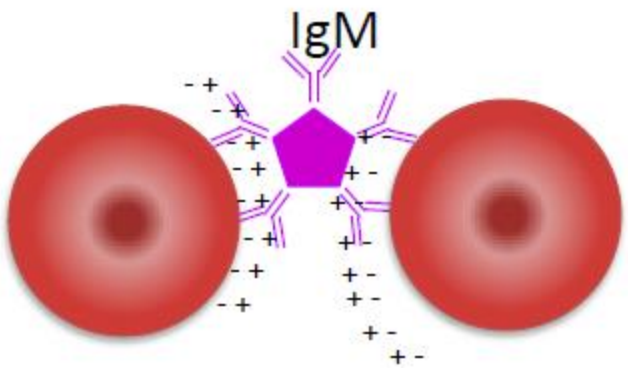
Test Theory

Unexpected/Immune AB binds known Antigen present on the surface of the Reagent Red Blood Cells.

The reaction is visible after the agglutination occurs.

IgM

Because it has 10 Antigen binding sites, IgM can bind between RBCs ⇨Direct Agglutination (the agglutination is visible)



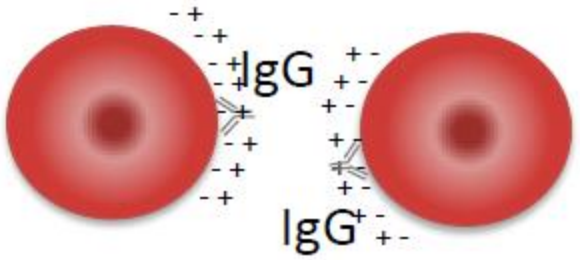
Test Theory

IgG

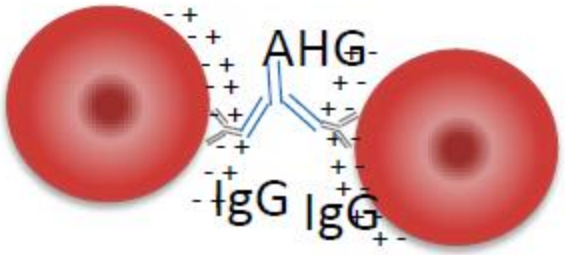
It cannot bind between RBCS. We need to add “artefact” to allow the agglutination

⇨ **Indirect Agglutination**

- The first step will be to fix the Ab to the Antigen.



- The second step to create a “bridge” between 2 RBCs.



Test Theory

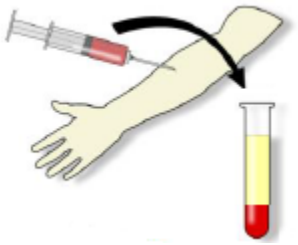
- **2 methods are available to detect immune AB:**

  - Indirect Anti-Globulin Test, IAT, called also Indirect Coombs Test, ICT.

  - Enzyme test. The latest test cannot be performed alone as some antigens are destroyed by enzyme (Antigen Fya, Fyb, M,N,S,s…)

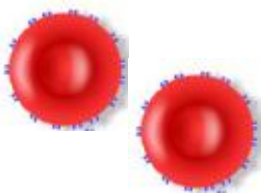
IAT: Components

– **I**ndirect **A**nti-human **G**lobuline Test/**I**ndirect **C**oombs **T**est



Patient plasma/serum containing unexpected IgG Antibodies

Reagent Red Blood Cells containing known Antigens

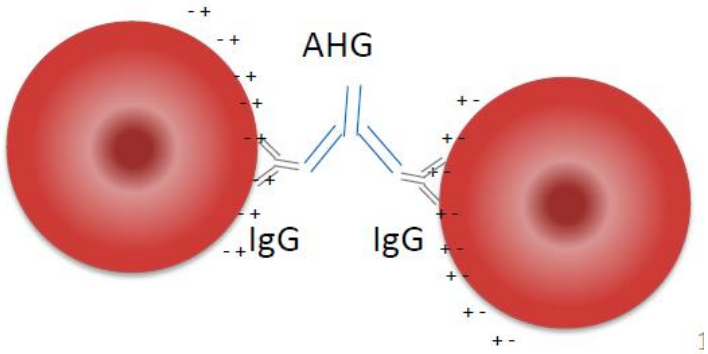


IAT: Components

BLISS
BioVue Low Ionic Strength Solution

- Blood cells have a slight negative charge.
- This charge can interfere with the binding between antibodies, reducing agglutination.
- LISS (OCD product is “BLISS”) is used to reduce negative charge, for better agglutination
- RBCs diluted at 0.8% already contain the BLISS. It needs to be added only with 3% cells

Anti-Human Globulin (**AHG**)is produced from rabbits that are immunized with human serum. They produce anti-human antibodies which will bind to human antibodies
Using AHG in blood testing will provide assistance to create the “bridge” between Antibodies from 2 cells allowing the agglutination.

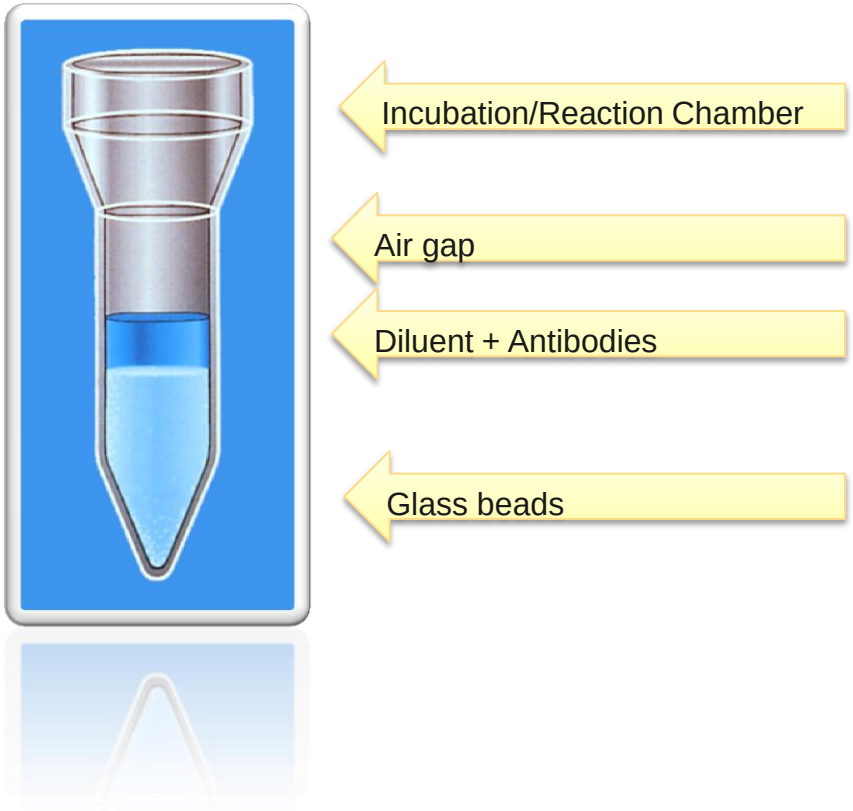


Column Agglutination Technology

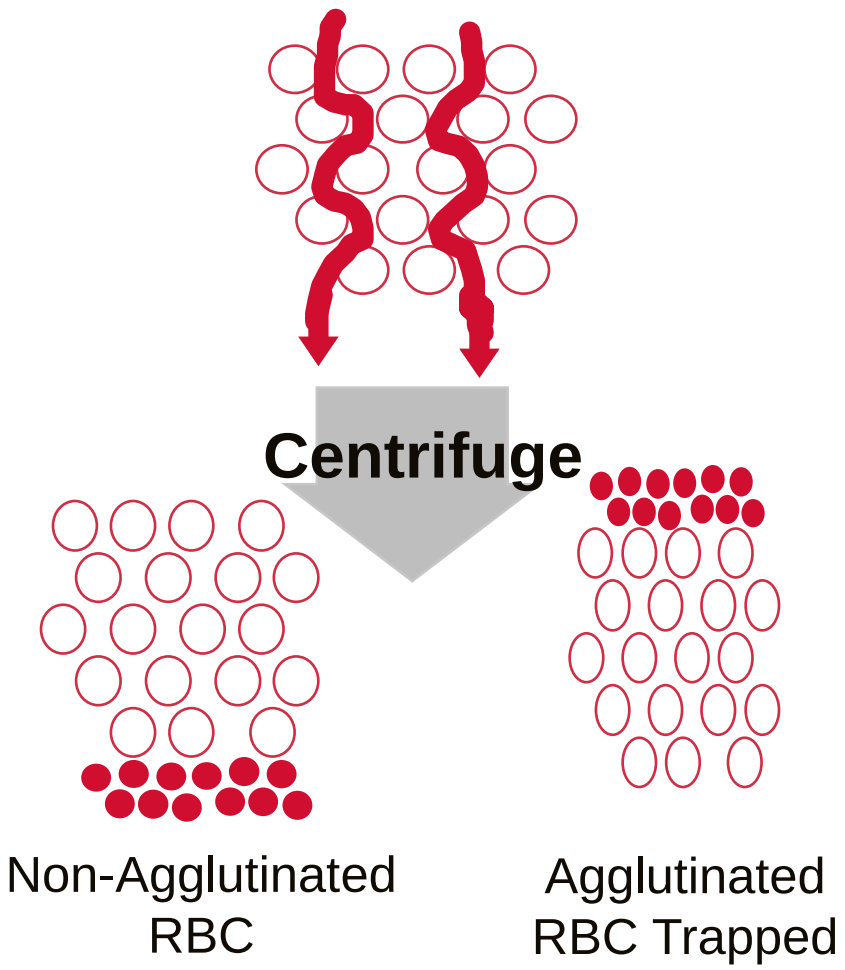
Screening and Identification:

Serum/plasma & reagents RBC & Bliss to be added to column

AHG reagent contained in the diluent of the cassettes (22/33)

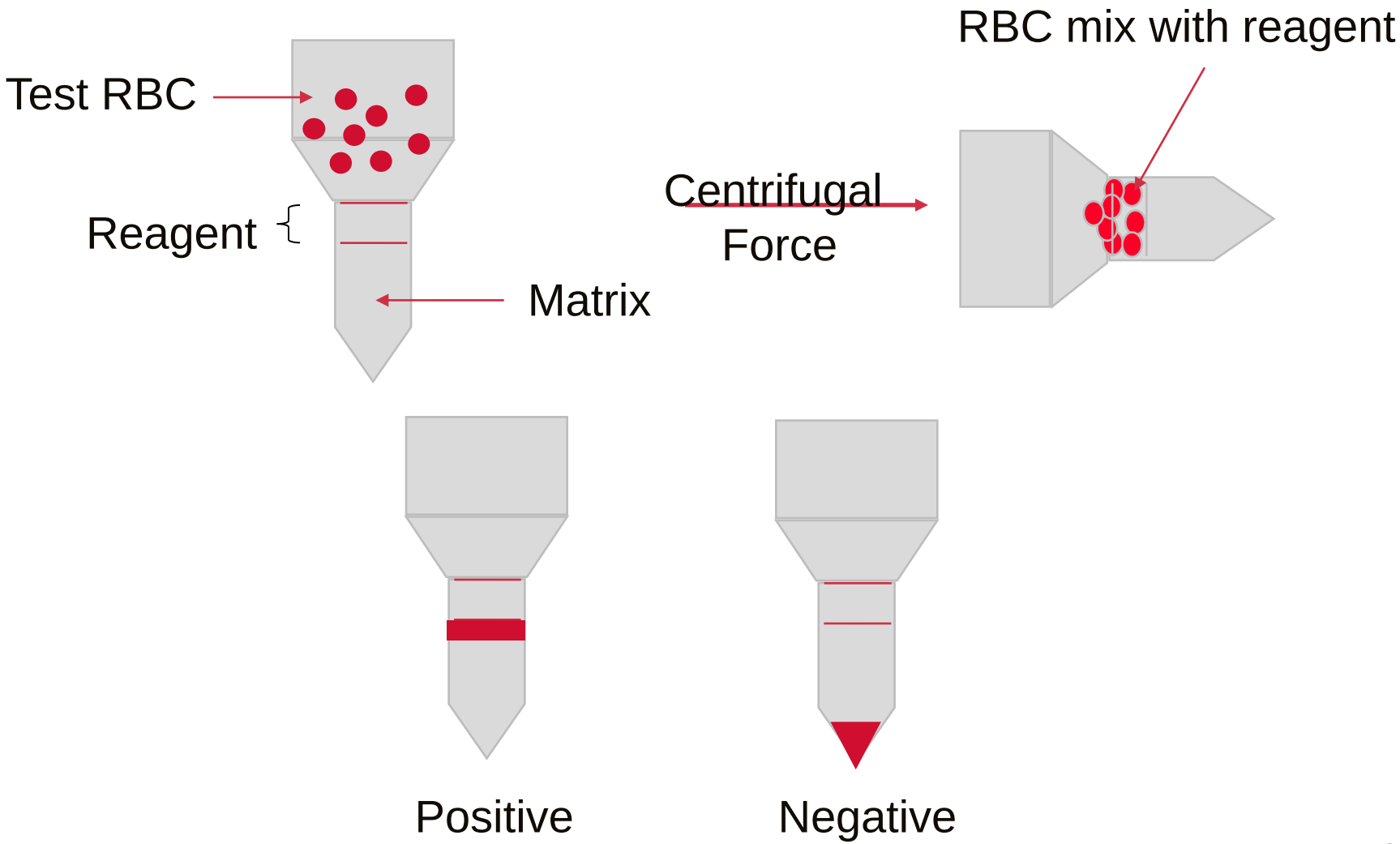


Column Agglutination Technology

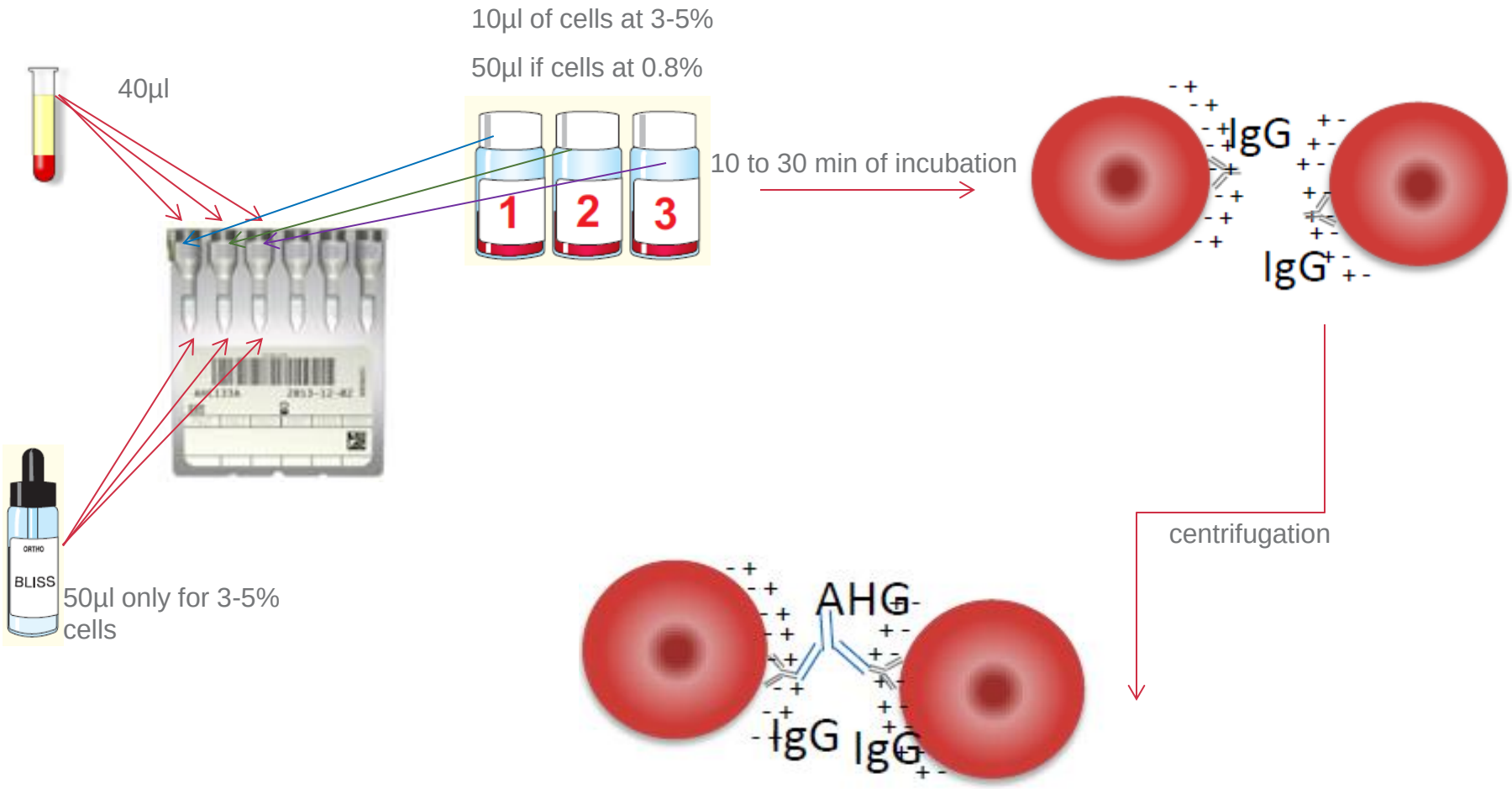


- The CAT uses defined volume of RBCs instead of drops of high cell concentration (as in tube method)
- The CAT is a standardised method (centrifugation vs shaking)
- The CAT is a stable support for multiple readers or for electronic reading
- Beads act as a filter because of : Size, shape and composition

Column Agglutination Technology



IAT Test



IAT Test

Cassette used for the IAT

- AHG anti-IgG (33)
- AHG Polyspecific (22)

Reagent used for the IAT test

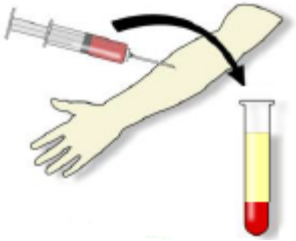
- Bliss if RBCs are diluted at 3-5%

RBCs used for the IAT test

- Selectogen at 3-5% or 0.8% ⇨2 cells
- Surgiscreen at 3-5% or 0.8% ⇨ 3 cells
- BioVue Screen Ficin at 3-5% or 0.8% ⇨3 untreated cells

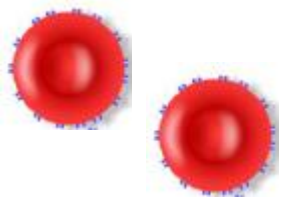
Enzyme Test : components

- **Enzym test**



Patient plasma/serum containing unexpected IgG Antibodies

Reagent Red Blood Cells contain known Antigens and are treated with Papain, ficin or bromelin



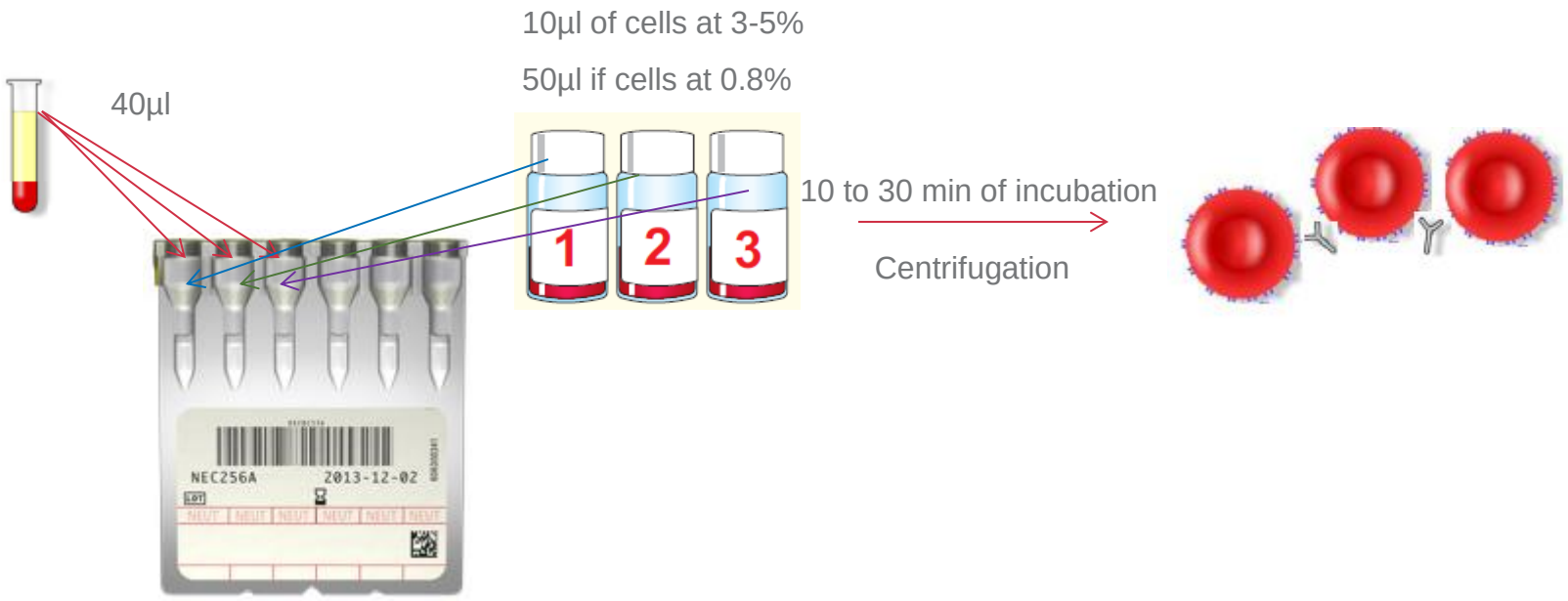
Enzyme decreases the potential zeta and allow the agglutination of cells

Enzyme destroys some antigen like: Fya, Fyb, S,s,M,N..

Some Ag-Ab reactions can be enhanced by enzyme, like D, C, E, c, e…

Neutral cassettes contain only PEG diluent

Enzyme test



Enzyme test

Cassettes used for the enzyme test

- Neutral (88)

RBCs used for the enzyme test

- BioVue Screen Ficin at 3-5% or 0.8% ⇨3 treated cells

Always performed as a second intention test

Antigens destroyed : Fya, Fyb, M, N, S, s

enhanced: Rh, Kell

Antibody Screening Test Interpretation

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Reading

Different Reaction Grades

4+ A band at the top of the beads

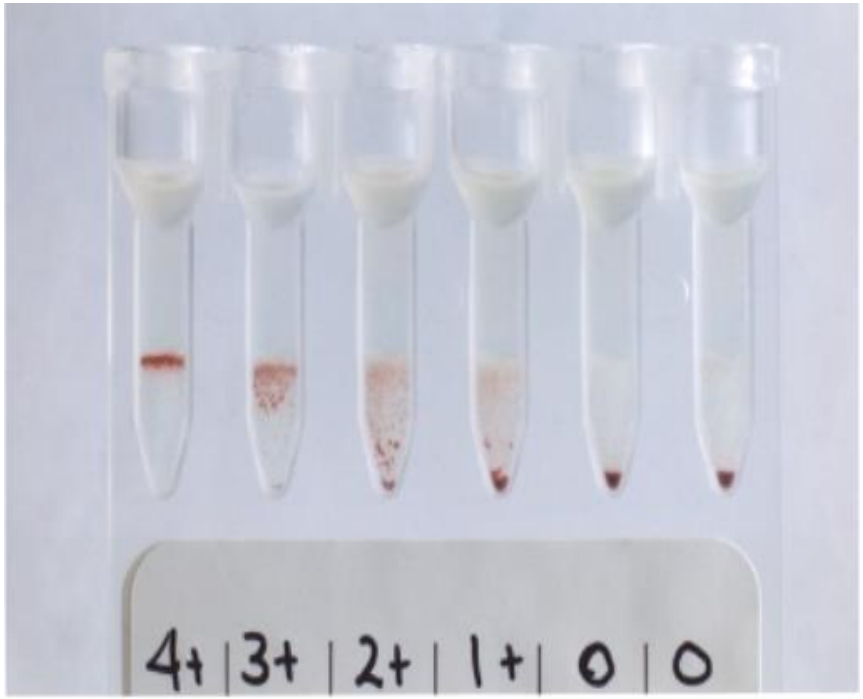
3+ Most RBC remain in the upper half of the beads column

2+ Agglutination throughout the length of the column. Small cell button

1+ Agglutinated cells remain in the lower half. A button cells is present

0.5 Disrupted bottom + small agglutination above the button

0 Negative : All RBC passed through the glass beads



Antibody Screening

Negative : absence of unexpected antibodies represented in the screening panel

Validity : 3 days (72hours)

Can be extended to 21 days under certain conditions (absence of transfusion, pregnancy, transplant in the last 6 months)

Antibody Screening

Result positive : presence of unexpected antibodies

perform an identification

- Unexpected red cell alloantibodies can be found in 1% to 2 % of the population
- RH (anti D, anti E) Kell (anti K)

- Negative Crossmatch required prior any transfusion

			Rh-hr								KELL						DUFFY		KIDD		Sex Linked	LEWIS		MNS				P	LUTHERAN	
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
1	R1wR1	315357	+	+	0	0	+	0	+	0	0	+	0	+	/	+	0	+	0	+	+	0	+	+	+	+	0	+s	+	+
2	R2R2	319426	+	0	+	+	0	0	0	0	0	+	0	+	/	+	+	+	0	+	+	+	0	+	0	+	+	+	0	+
3	rr	102926	0	0	0	+	+	+	0	0	+	+	0	+	0	+	+	0	+	0	+	0	+	0	+	0	+	+s	0	+
	Patient Cells																													

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

LOT NO.
8SS363

EXP. DATE
2017-12-26
CCYY-MM-DD

Ortho Clinical Diagnostics

Reagent Red Blood Cells
0.8% Surgiscreen®

ANTIGRA
Antigen
Profile

635200771

Antibody Screening

- Mandatory prior any transfusion
- Post transfusion : between 3rd and 5th week
- During pregnancy

Difficulties

- **No match / incomplete match**
- **Weak antibodies**
- **Antibody against low frequency antigens**
- **Pan agglutination/ auto antibodies**
- **Multiple antibodies**

Questions?

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Thank you!

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