The Discovery and Significance of Selected Blood Groups

Marion E. Reid, PhD, DSc (Hon.)

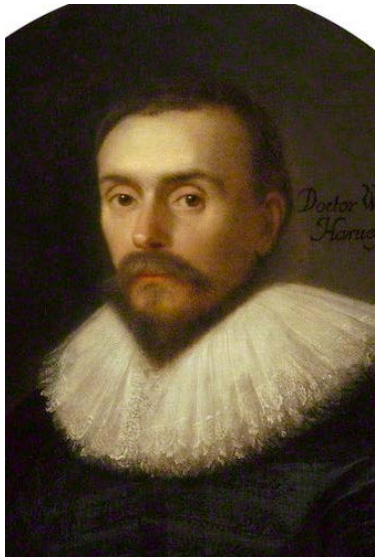
James Blundell Award Lecture

BBTS Annual Conference
September 2014 – Harrogate

Talk Overview

- **James Blundell: improved equipment used for direct blood transfusions; showed the importance of matching species**
- **For predictably safe transfusions, matching for ABO - and other blood groups - was needed**
- **How techniques have evolved over my career to allow discoveries, illustrated with two blood group systems – DO and JR**

Background



William Harvey
1628 theory that blood circulates

Richard Lower
1666 transfused blood from animal to animal/human, intravenously

Jean-Baptiste Denys
1667 patient died from animal transfusion or arsenic?!

By the end of the 17th Century, blood transfusion was prohibited and the practice was abandoned until the 19th Century

Images courtesy Wikipedia

James Blundell, MD (1790-1878)

Graduated with MD from University of Edinburgh in 1818.

Moved to St. Thomas's, London and became Professor of obstetrics and physiology at Guy's Hospital.

Image courtesy Wikipedia

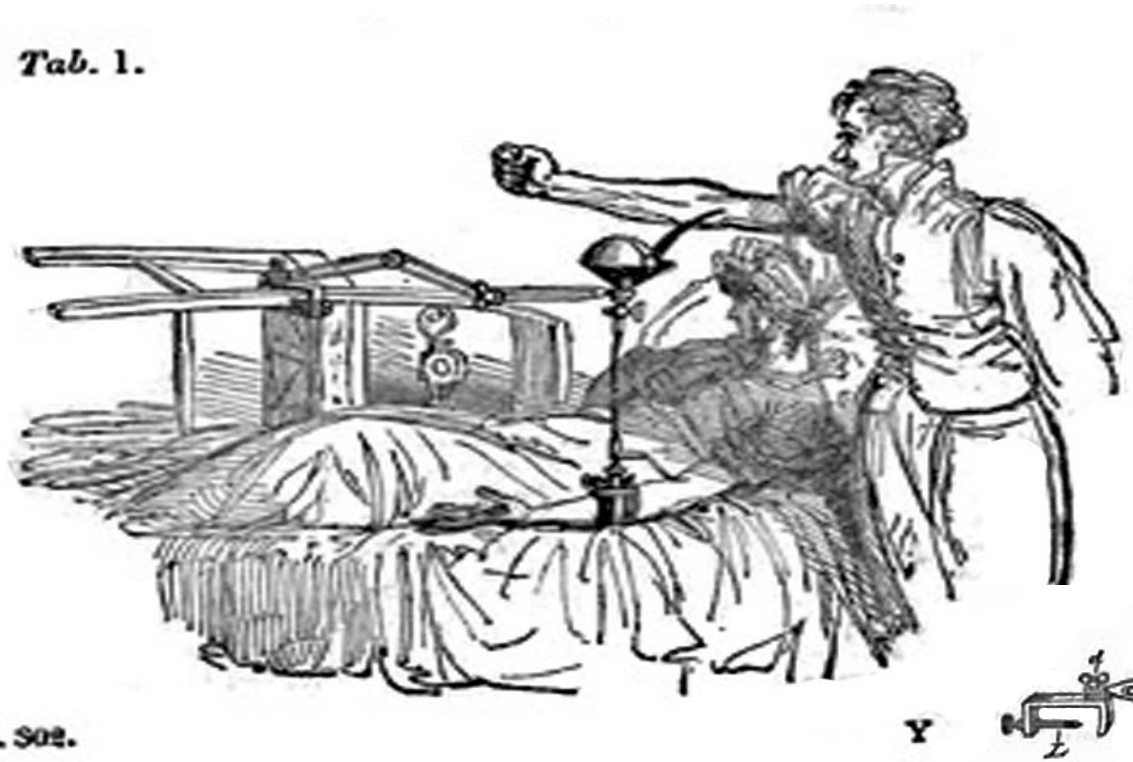
Blundell and Blood Transfusion

- **Despite the ban, Blundell bravely performed a series experiments with animals, including, largely unsuccessful, dog to human transfusions**
- **In 1818, he transfused a dying patient with human blood, thereby showing feasibility**
- **Many of his patient's died, mainly because those he chose were unlikely to have survived even if transfusion had been successful. Like many of his contemporaries, Blundell felt that transfusion could have a restorative effect, even after death.**
- **Despite his initial failures, Blundell persisted and in 1825, performed his first successful human to human transfusion to a woman who had severe postpartum bleeding**

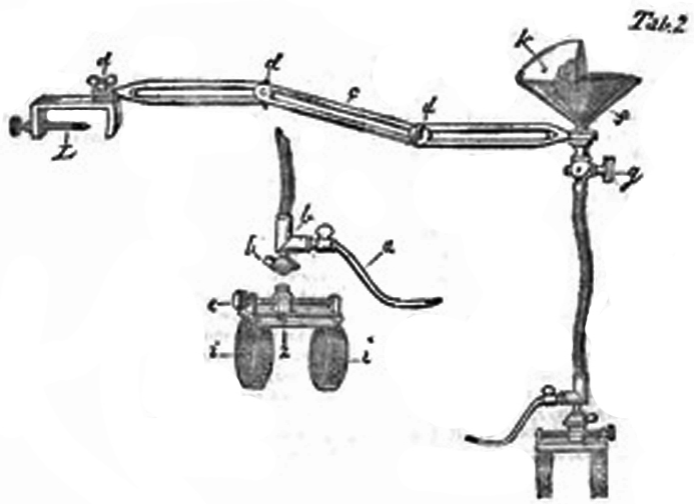
Blundell and Technique/Equipment

- He used a cannula of his own design to connect an artery in the donor's arm directly to the patient's vein. A cut was made to expose the blood vessels before they could be connected—a difficult and messy affair.
- His gravitator allowed blood to be transferred from donor to patient without the need for surgical exposure of the blood vessels so that the donor's and patient's blood vessels could be used repeatedly, and the flow could be regulated
- The quantity of blood transfused could be measured
- He also pioneered the transfer of blood from donor to patient using a syringe, showing that blood could be held briefly outside the body, and his impeller allowed blood to be transfused under pressure

Blundell's Gravitator



Gravity-fed apparatus described in 1829 in the Lancet



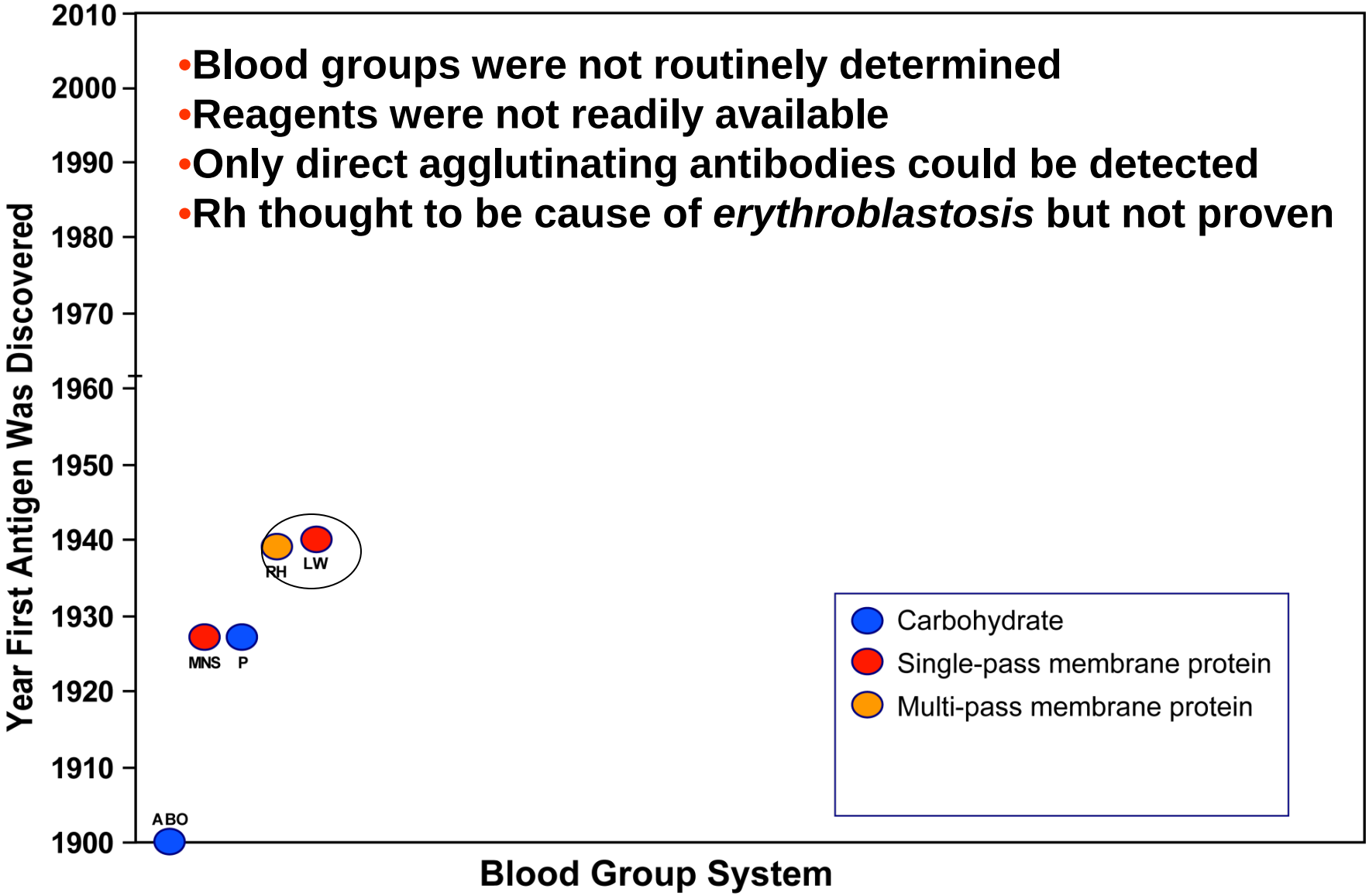
Held upright by clamping it to a chair placed on the patient's bed!

Importance of Matching Blood Groups

- **While such improvements in equipment were important in the advancement of direct transfusions, they did not ensure a predictable safe outcome. Some patients tolerated the blood while others experienced severe reactions.**
- **At that time, it was thought that blood from healthy people was all the same; but in 1900, using hæmagglutination, Landsteiner demonstrated this was erroneous**
- **Amazingly (to us today) it took over two decades before the importance of ABO blood groups in transfusion was realized and pre-transfusion testing became commonplace**

Karl Landsteiner (1868-1943)

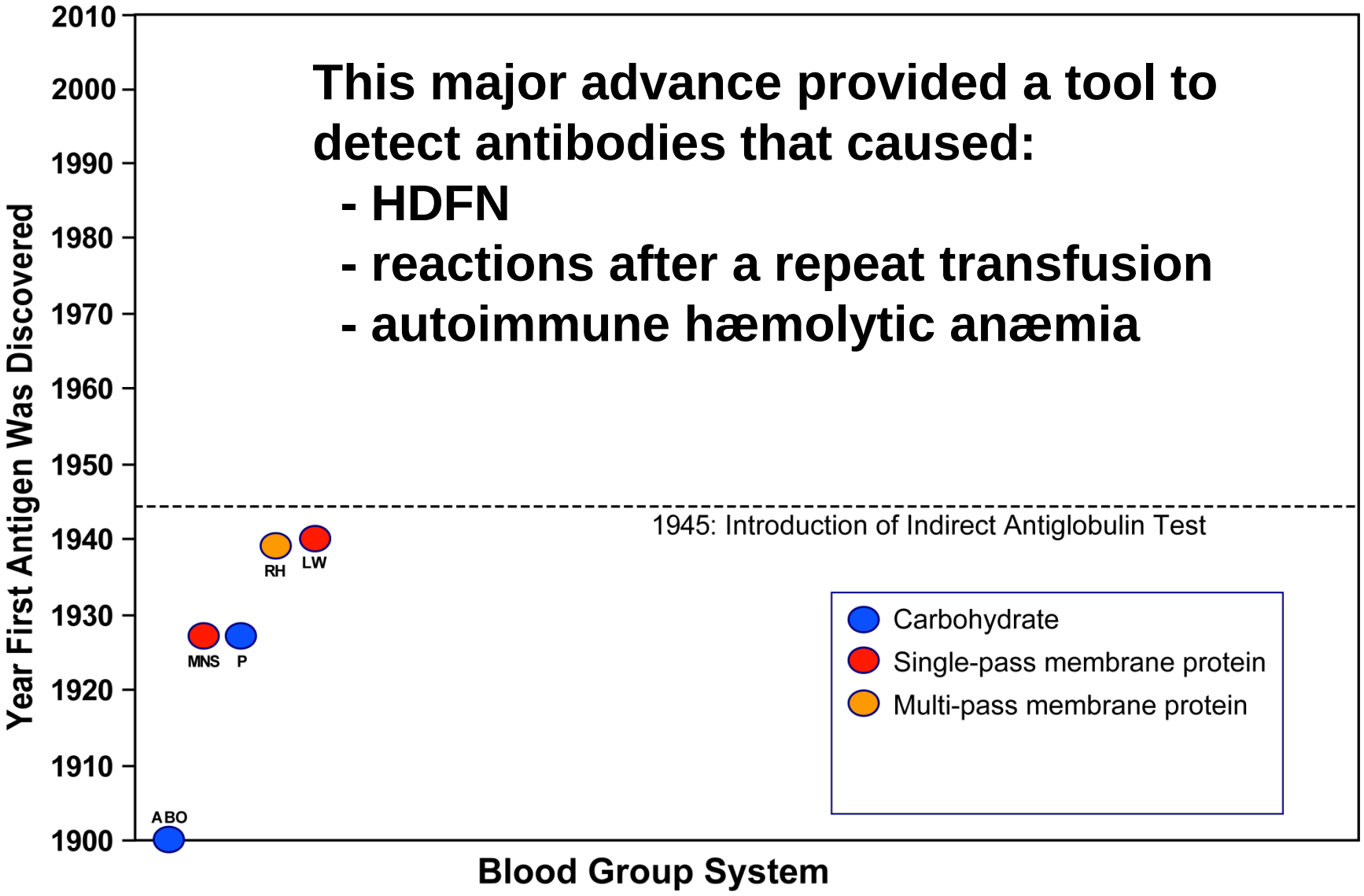
1944


•Blood groups were not routinely determined
•Reagents were not readily available
•Only direct agglutinating antibodies could be detected
•Rh thought to be cause of *erythroblastosis* but not proven
Year First Antigen Was Discovered
2010
2000
1990
1980
1970
1960
1950
1940
1930
1920
1910
1900
RH
LW
MNS
P
ABO
Carbohydrate
Single-pass membrane protein
Multi-pass membrane protein
Blood Group System

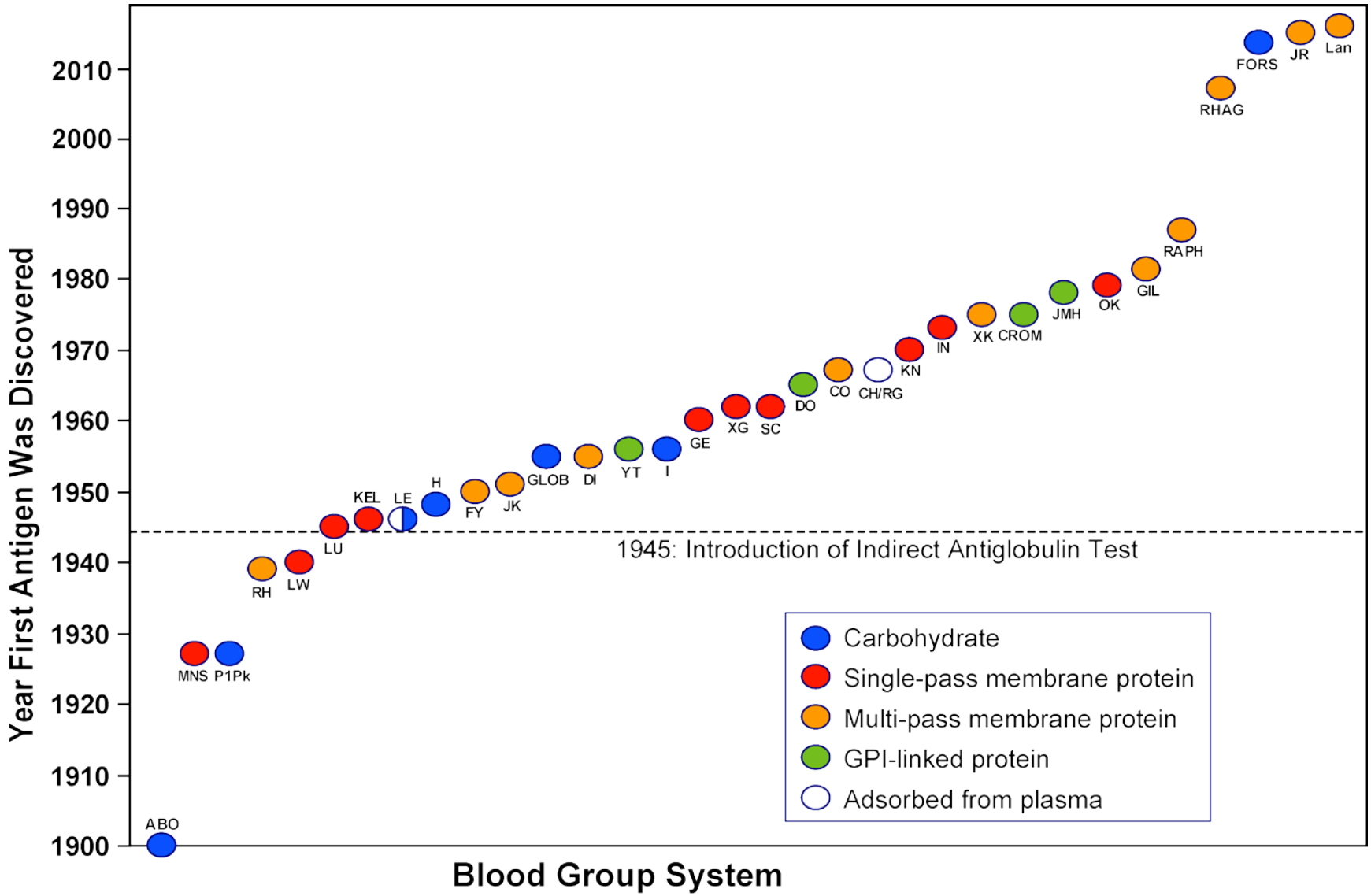
Transfusion Becomes Commonplace

- During World War II, the value of transfusion became apparent
- Thereafter, the number of transfusions administered to civilians increased and repeat transfusions became commonplace
- As a consequence, the number of incompatibilities caused by blood group antibodies increased dramatically
- Recognition that HDFN was caused by anti-D led to the investigation of other cases of HDFN and identification of even more blood group antibodies

1945: Indirect Antiglobulin Test



Blood Group Systems 2012 (2014 VEL & CD59)

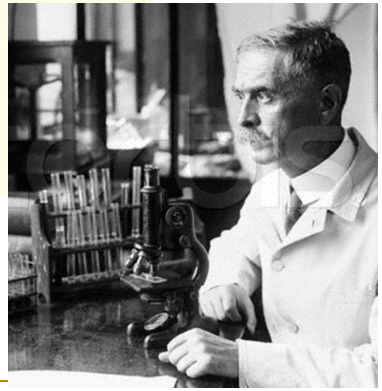


1953: Watson & Crick said DNA is a double helix

1960s Immunohæmatology Work Station

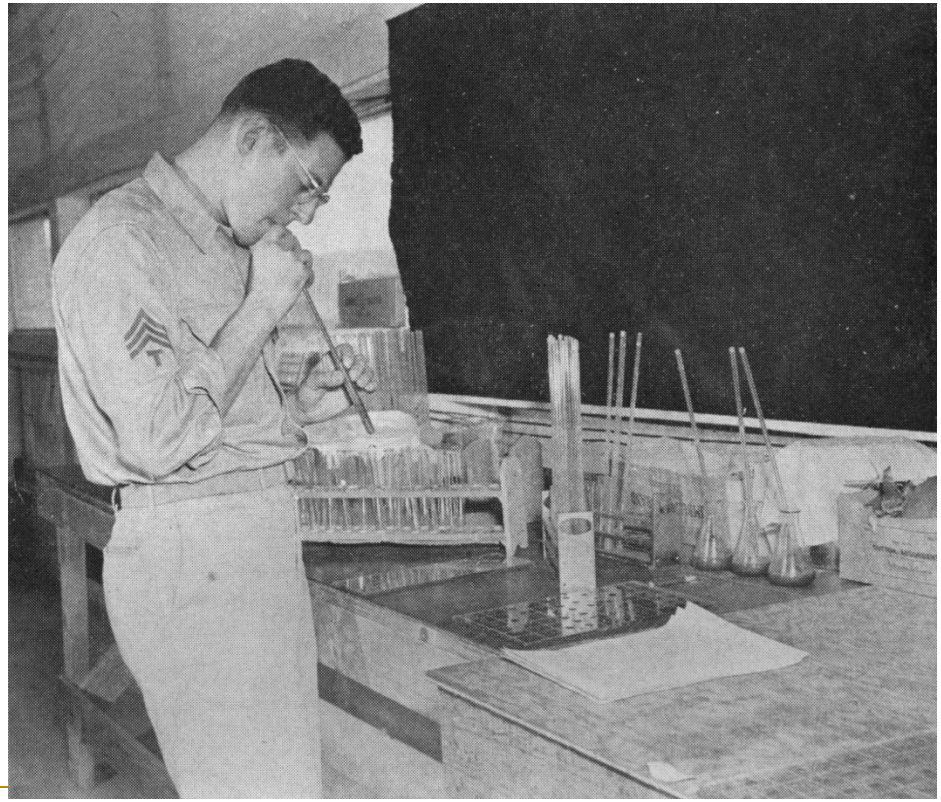
1. Angle centrifuge.
2. Beaker containing isotonic saline solution.
3. Block holding precipitin tubes.
4. Bottles of saline solution.
5. Calculating machine.
6. Discarded slide container.
7. Pasteur pipette.
8. Pipette stand.
9. Protocol for recording results of tests.
10. Punched card with which statistical analysis of results are made.
11. White tile.
12. Pipette stand.

Plate 1 Interior of a Blood Group Research Laboratory.

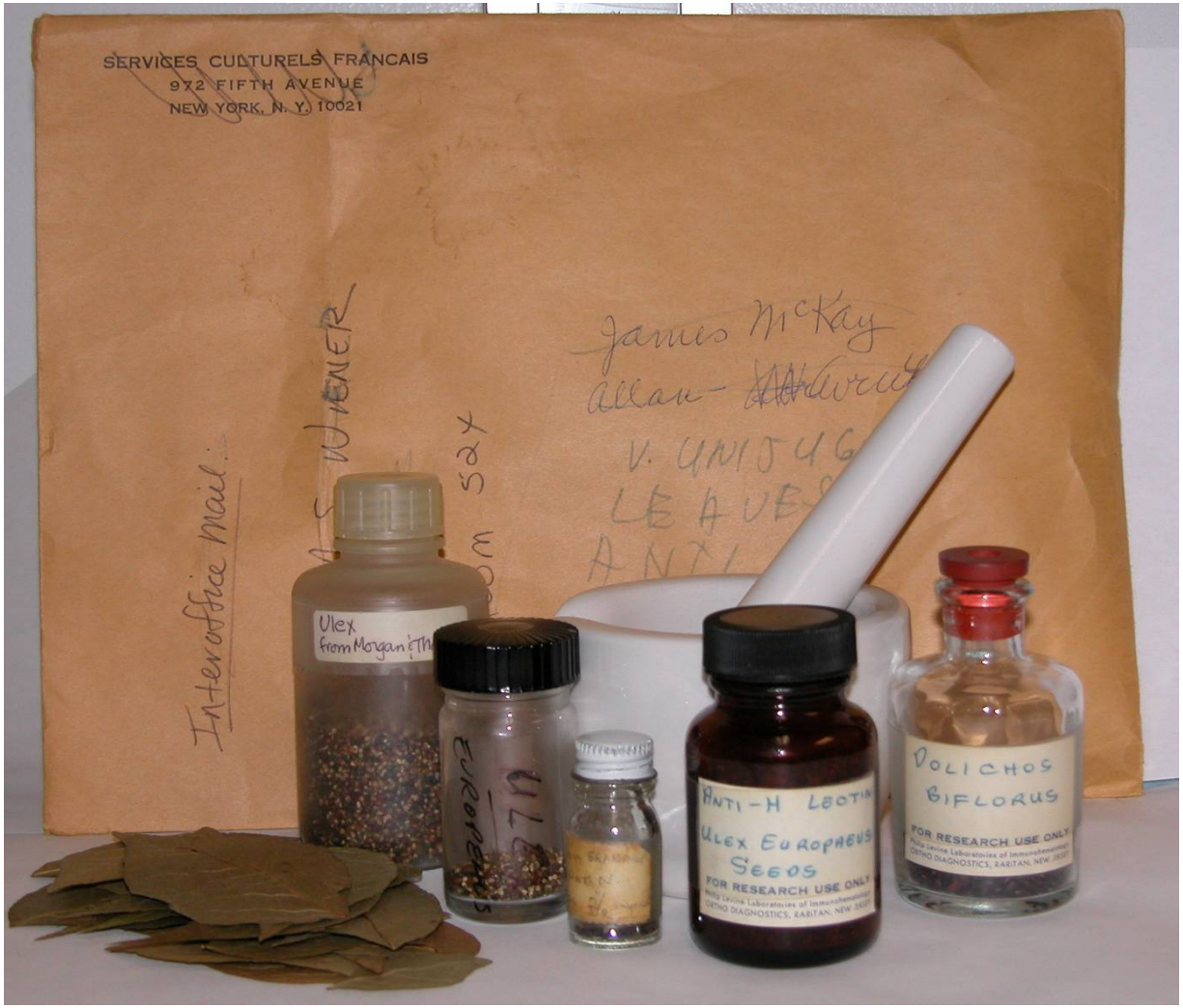


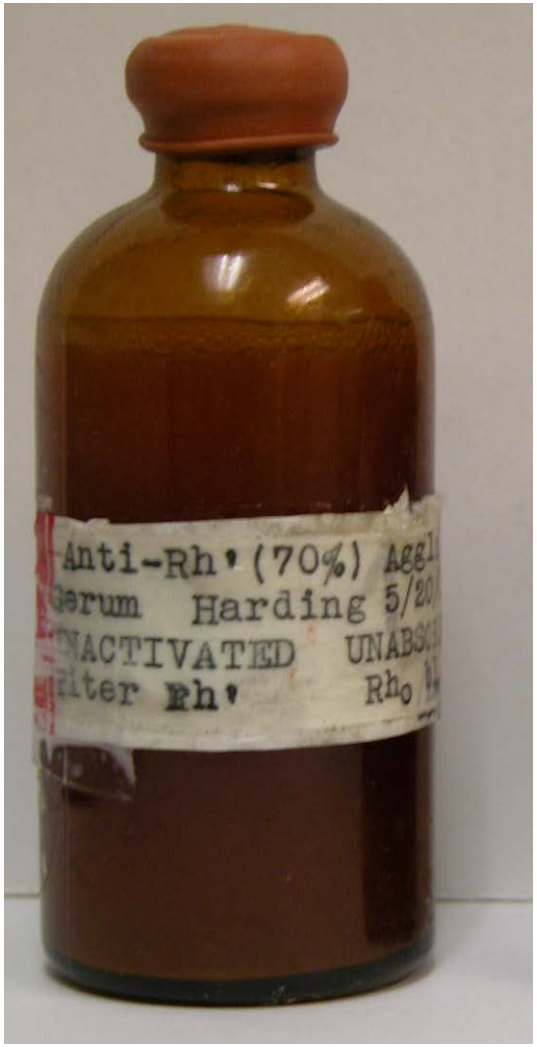
cGLP in 1960s and 1970s

- Tile or tube settle ABO
- No protective clothing
- Mouth pipetting



cGLP Reagents: Seeds, Leaves, and Plasma

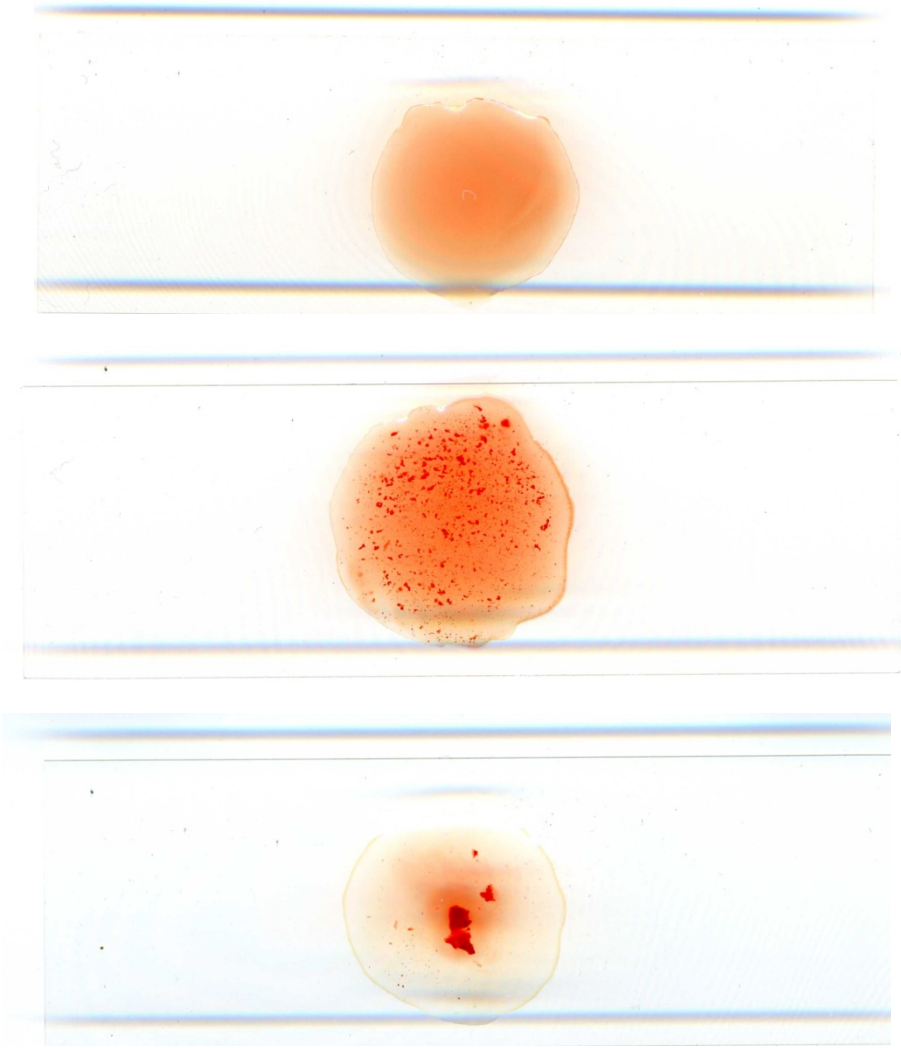


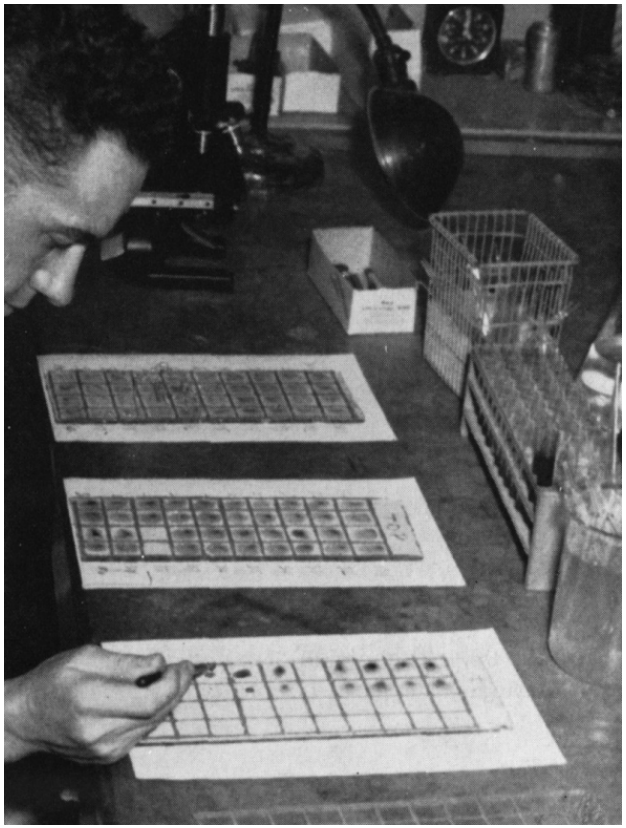


Not Available in the early 1960s

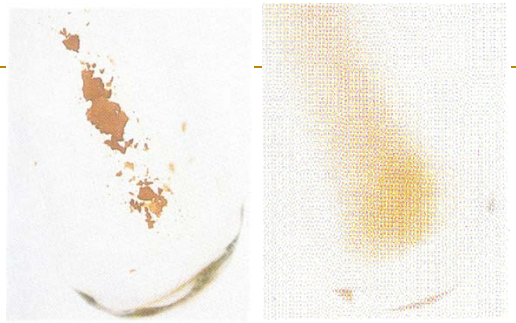
- Disposable glassware
- Commercial reagents
- Equality of salaries
- Testing blood for viruses
- Blood components
- Rh immune globulin
- Blood group gene cloning
- PCR and DNA analysis
- Photocopying machines
- Disposable pens
- Hand calculators
- Answering machines
- Cassette tapes
- Personal computers
- Hand-held hair driers
- Color TVs
- Beatles

Agglutination, or not, was the basis of all we did





Hæmagglutination



- **“Gold Standard” method to detect the presence of blood group antigens on RBCs**
- **Simple and, when done correctly, has a specificity and sensitivity that is appropriate for most testing**
- **For decades, direct and indirect hæmagglutination tests served the transfusion community well**

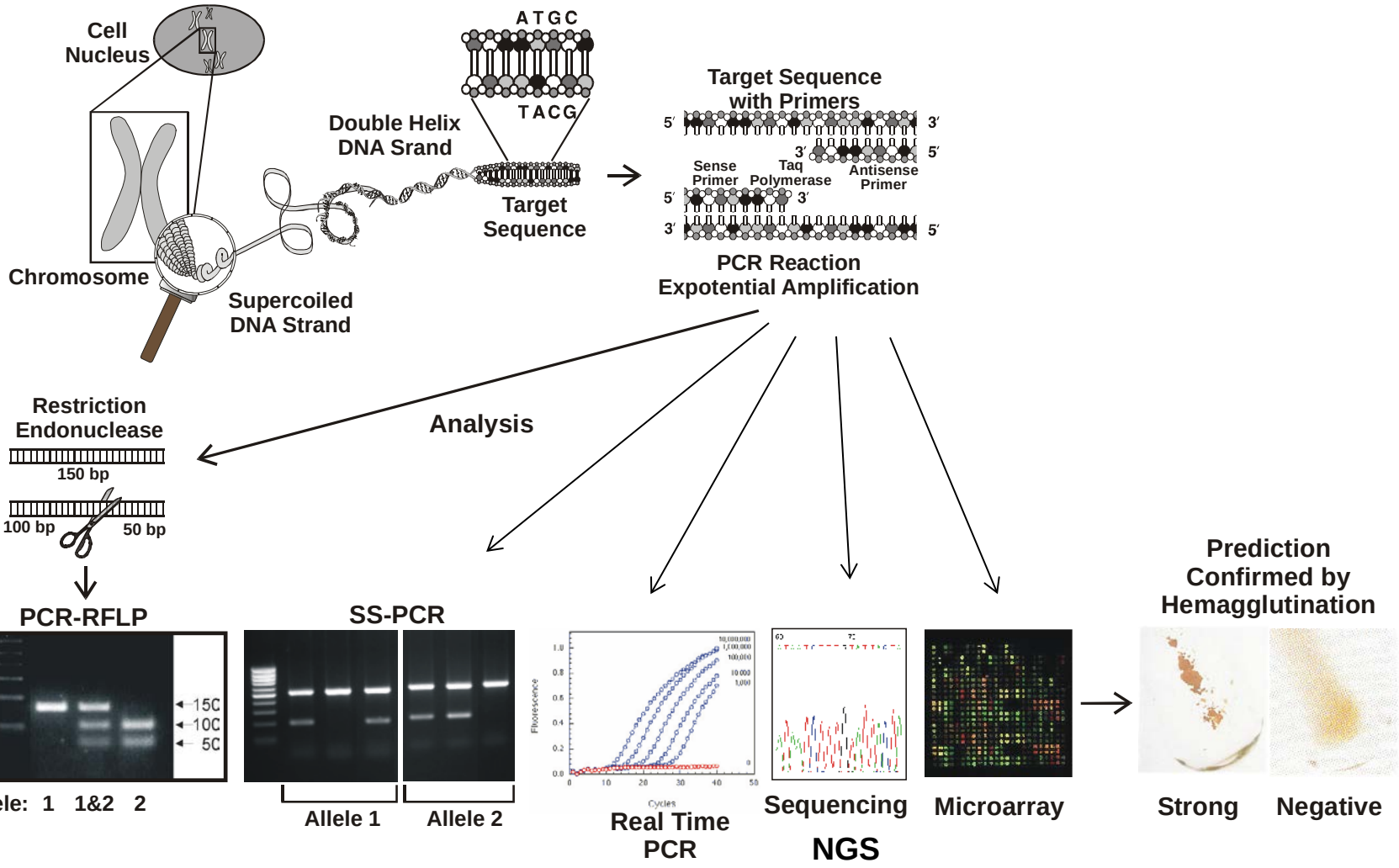
Hæmagglutination: Limitations

- Labor-intensive testing and data entry, so a relatively small # of donors can be typed for a relatively small # of antigens, which limited antigen-negative inventories
- Source material is diminishing
- Cost of commercial reagents is escalating
- Home-brew antibodies may be only partially characterized, limited in volume, weakly reactive, or not available
- Can be difficult to phenotype RBCs from a recently transfused patient, and RBCs coated with IgG
- Can be difficult to distinguish an allo from auto antibody
- May not reliably determine zygosity
- An indirect indication of a fœtus at risk of hæmolytic disease of the fœtus and newborn

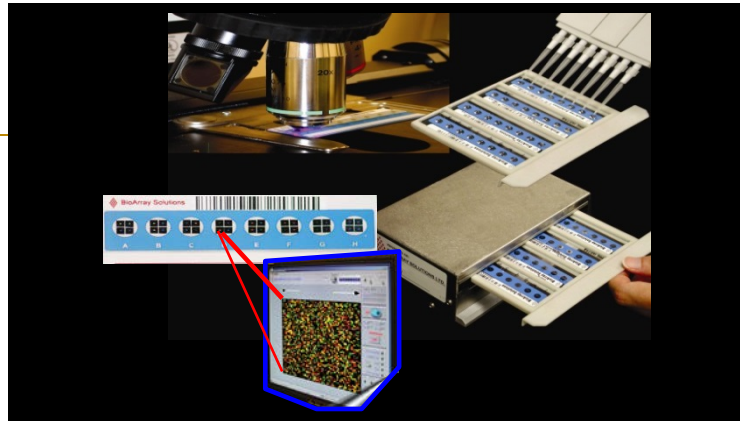
Developments in DNA Testing

Decade	Effect
1960s	Genetic code for synthesis of proteins from DNA/RNA was defined
1970s	Reverse transcriptase Genes are not continuous segments of coding DNA but are usually interrupted by non-coding segments
1980s	Polymerase chain reaction Some human genes were cloned, including 1st blood group (*GYPA*/MNS)
1990s	Prediction of blood groups by laboratory developed DNA-based tests
2000s	DNA arrays for predicting blood groups

Nucleated Cell to Red Cell Antigens



DNA Arrays



Cloning and sequencing genes encoding the 35 blood group systems and determining the molecular bases of most blood group antigens and phenotypes has made it possible to predict a blood group antigen of a fœtus at risk for hæmolytic disease and anæmia, and mass screen for antigen-negative blood donors.

****

- **PreciseType HEA (Immucor) predicts 35 RBC antigens from 11 blood group systems simultaneously**
- **RHD and RHCE arrays**
- **Next generation sequence (NGS)**

HDFN Prediction by DNA Typing: Value

If fœtus is predicted to be:

- **antigen-positive: should monitor as usual**
- **antigen-negative: no need to monitor**

When the father is a heterozygote, predicting a blood type is of value in 50% of pregnancies because traumatic, expensive, invasive procedures are not needed

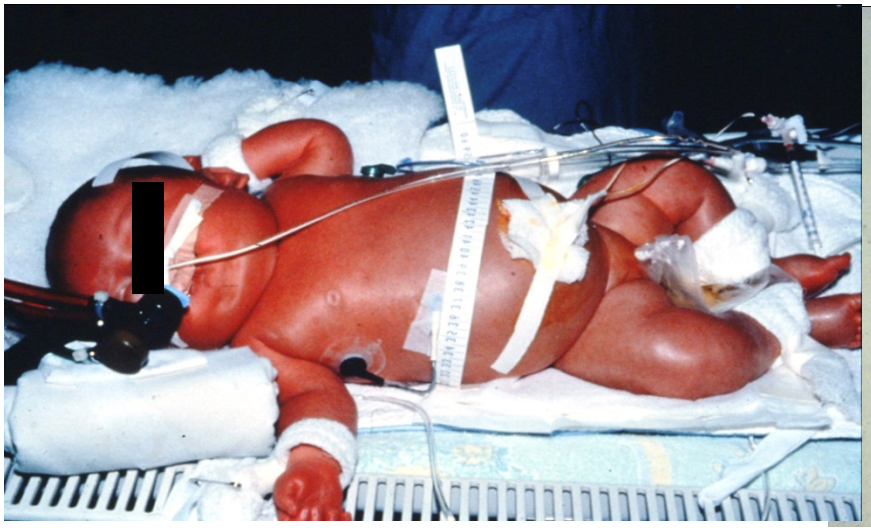
DNA Testing for HDFN Due to Anti-K

DNA testing is valuable to determine an at-risk pregnancy due to anti-K because:

- **The titer of anti-K is not predictive of HDFN**
  - **Low titer: severely affected fœtus**
  - **High titer: K– fœtus**
- **The bilirubin level in amniotic fluid is not predictive**
- **This is due to suppression of erythropoiesis as well as to immune destruction**

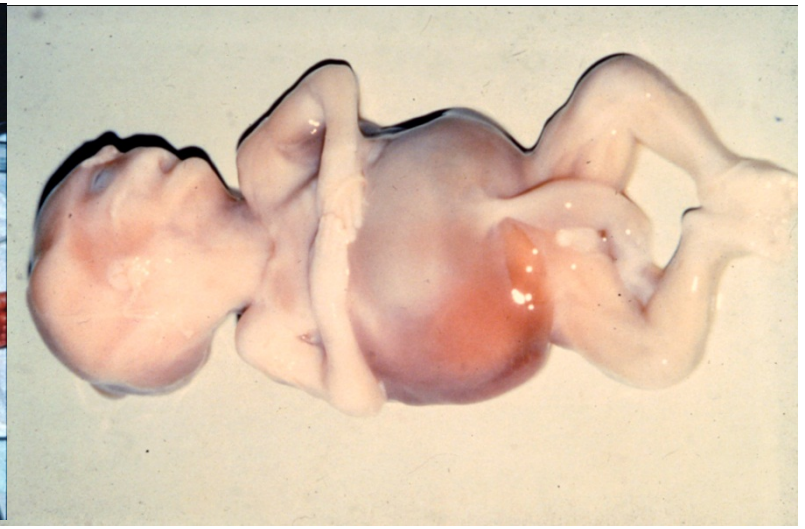
HDFN due to Anti-D and to Anti-K

Anti-D



Hydropic

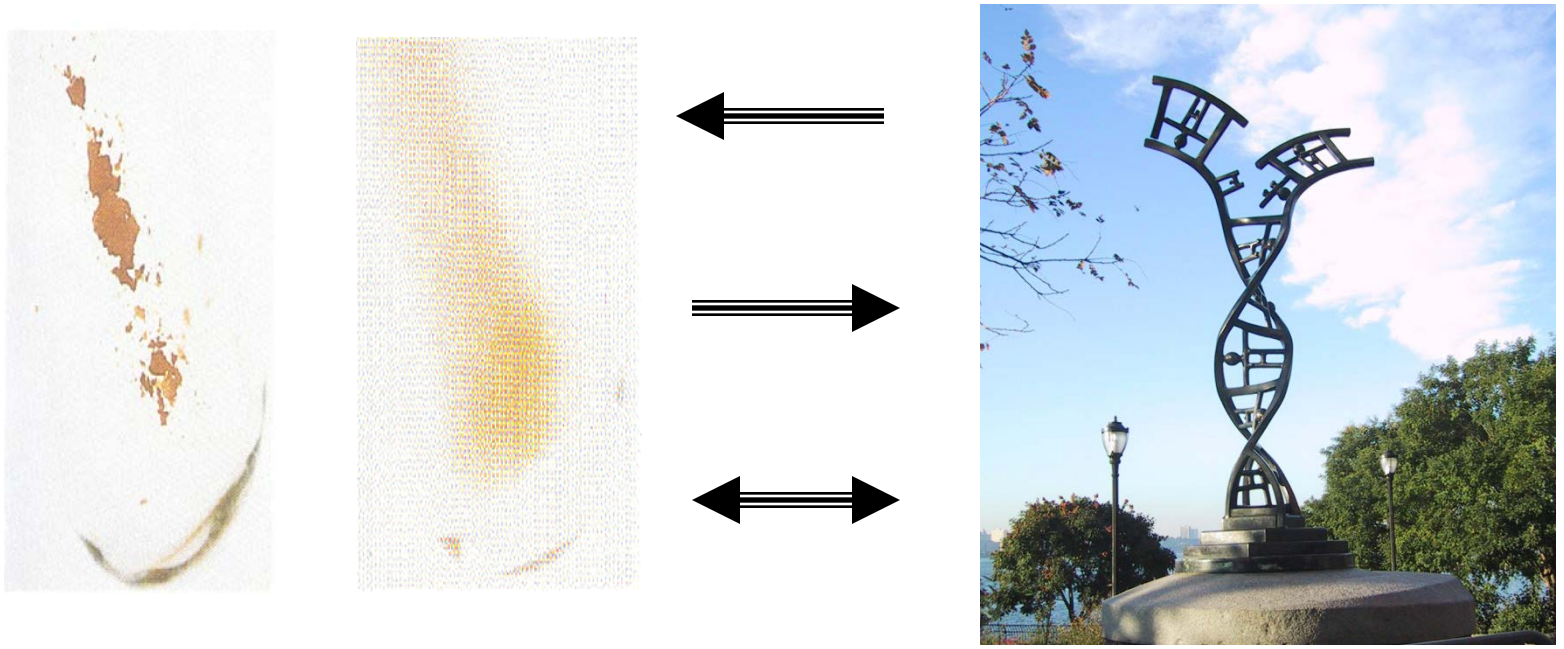
Anti-K



Hydropic and anemic

Thanks to Greg Denomme for photographs

Blood Typing for RBC Antigens



Hæmagglutination and DNA-based testing complement each other

Personal Experience

Role of techniques in discoveries illustrated with two blood group systems – DO and JR

Developments in Blood Group Techniques

1950-1960 Structural analysis of carbohydrate antigens (A, B, H, Le)

Hæmagglutination

1970-1980 Structural analysis of P, P1, Pk, M, N

SDS-PAGE

Monoclonal antibodies

1990-2000 Western blot analysis

Immuneprecipitation

Automated amino acid sequencing

DNA-based technology (PCR)

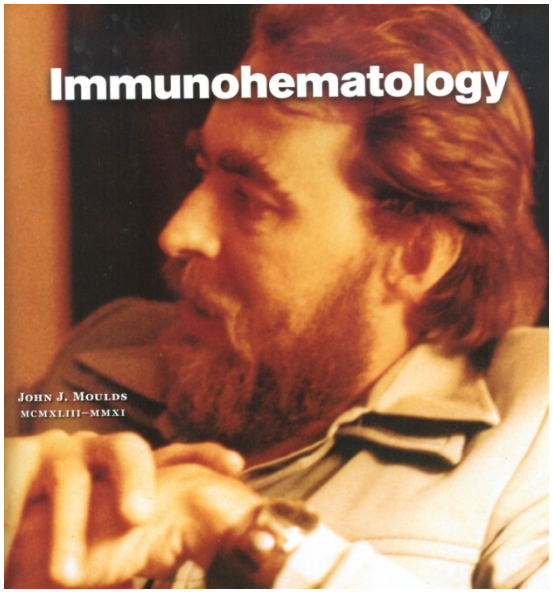
Dombrock Blood Group System

Over 50 years of study

Dombrock: Hæmagglutination (1960s & 1970s)

- Characterisation of the antigens and antibodies
- Relationship of Hy to Gy^a
- Gy(a–) RBCs lack Hy and Jo^a (null phenotype)

Sandy Ellisor



John Moulds

Joyce Poole

Dombrock: Immunoblotting (1980s)

- **Characterization of Dombrock glycoprotein**
- **Do is attached to the RBC membrane by a glycosylphosphatidylinositol (GPI) anchor. This was also shown (Telen, *et al.*) by passing RBCs from patients with PNH through a column coated with anti-DAF and typing the PNHIII RBCs that did not bind to the antibody.**

Fran Spring

Dombrock: Identifying Gene and Alleles

- ***In silico* analysis (1990s)**
  - **lead to cloning and sequencing of the gene (Jeff Miller)**
- **Manual PCR-based assay (1990s)**
  - **identified the nucleotide changes associated with the antigens**
  - **provided a way to screen for antigen-negative donors**
  - **identified several new alleles**
- **DNA Arrays (2000s)**
  - **provides a means to do high-throughput testing**



Transfection and Hybridoma Technologies (1990s)

- **Transfection of cultured cells provided a tool to study protein expression**

- **Transfected cells and synthetic peptides used as immunogens, followed by hybridoma technology, to produce many monoclonal antibodies**

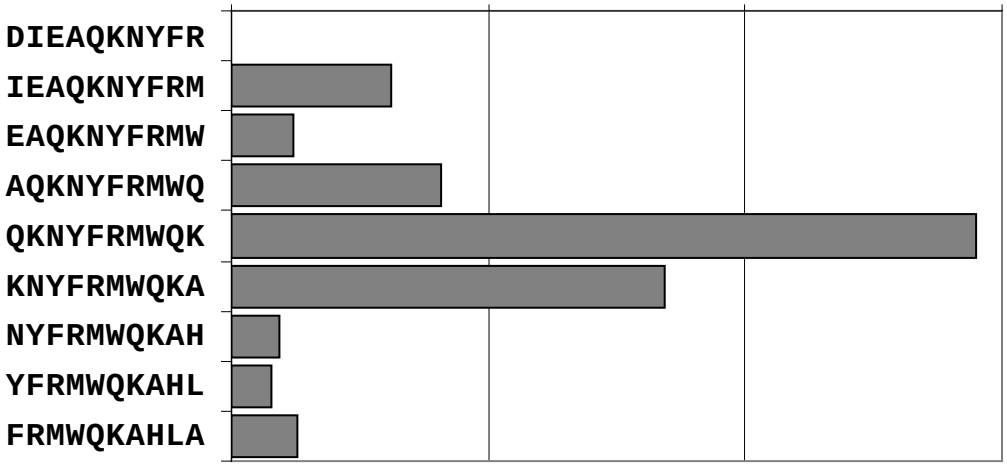
Karina Yazdanbakhsh

Pepscan Analysis (2000s)

- **Pepscan analyses using overlapping peptides (pin technology) allows precise epitope mapping of monoclonal antibodies**

Elwira Lisowska

Binding of clone 7C8 Mab to Dombrock ^{86}DIEAQKNYFRMWQKAHLA^{103} dekapeptides



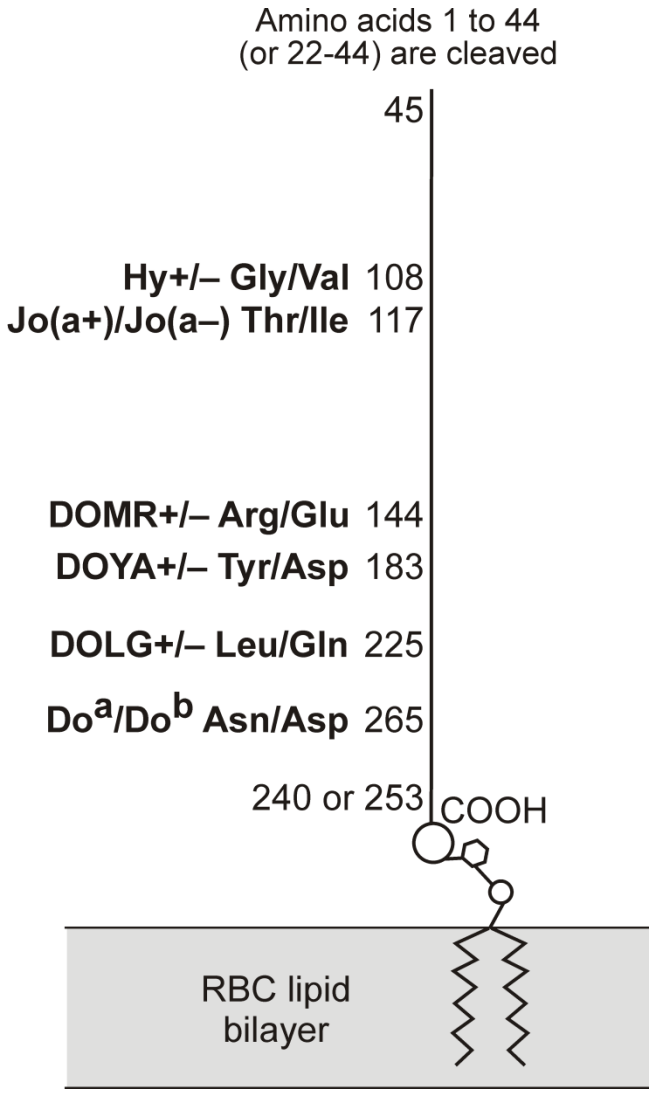
Epitope:

A Q K N Y F R M W Q

Q K N Y F R M W Q K

K N Y F R M W Q K A

Do Blood Group System



Gy^{a}

JR Blood Group System

Over 40 years of study

JR Background

- First described in 1970; named after Rose Jacobs, one of the first six probands
- Family studies showed the Jr(a−) phenotype is inherited as an autosomal recessive trait
- In 1990 Jr^a was placed in the ISBT 901 Series of High-Incidence Antigens: #901005
- The Jr(a–) phenotype has been found in people of northern European ancestry, Bedouin Arabs, a Mexican, and more commonly in Asians; notably Japanese (1 in 58 in the Niigata region)

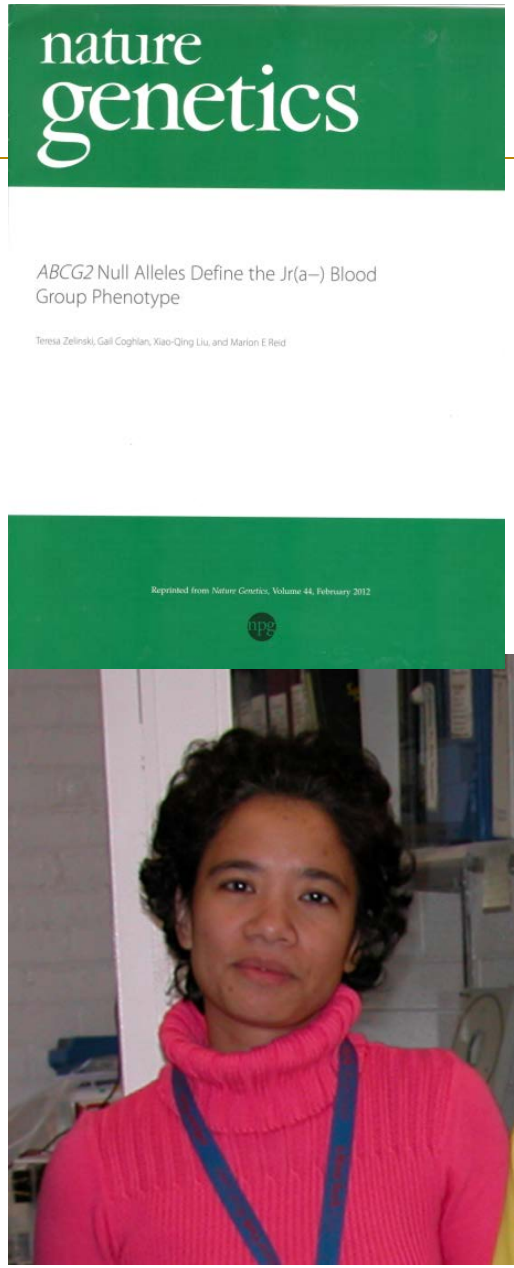
Clinical Significance of Alloanti-Jr^a

- **Transfusion reaction**
  - **^{51}Cr cell survival studies indicated reduced RBC survival was possible**
  - **A patient with anti-Jr^a had rigors after 150mL of crossmatch incompatible blood**
- **HDFN**
  - **Positive DAT but usually no HDFN**
  - **One fatal case of HDFN**

Attempts to Define the Antigen

- For many years, numerous laboratories, using various techniques, failed to characterize the Jr^a antigen
- Attempts to immunoblot and immune-precipitate the antigen using human anti-Jr^a were unsuccessful
- Homozygosity by Descent (HBD) gene mapping provided the key to identify the gene encoding the protein carrying the Jr^a antigen

Terry Zelinski



Kim Hue-Roye

Gail Coghlan

Homozygosity by Descent (HBD) Gene Mapping

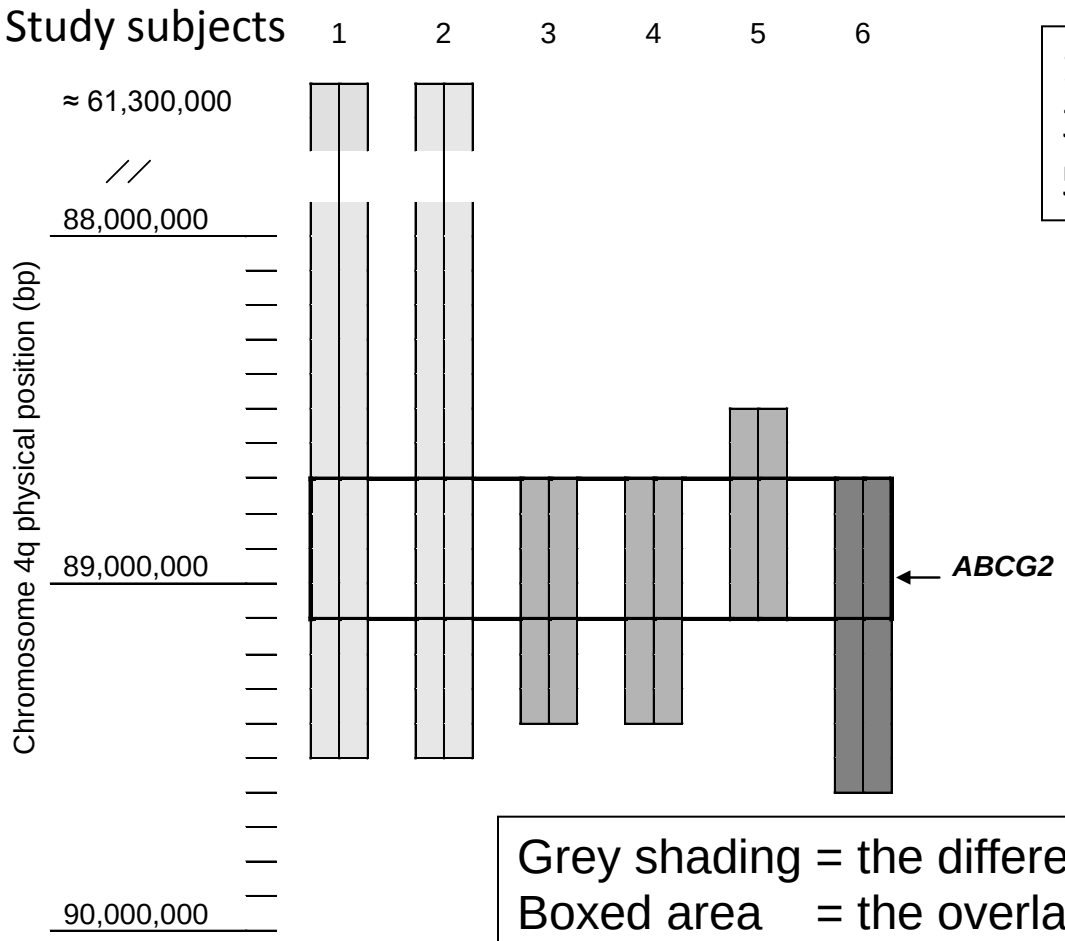
- **Genomic DNA from the 6 Jr(a–) subjects was first analysed for SNPs on an array (Affymetrix GeneChip Human Mapping 250K *NspI* array) and then by HBD**

Samples	Identically homozygous
Caucasian sisters	28.1 Mb
Asian siblings	682,000 bp
Asian - unrelated	397,000 bp with Asian sibs
Asian - unrelated	522,000 bp with Asian sibs

- **The minimal overlapping region was 397,000 bp**
- **This region was on the long arm of chromosome 4**

Zelinski *et al*., *Nat. Genet.* 2012;44,131

Regions of Homozygosity for Chromosome 4q in Jr(a–) Subjects



1,2 = Caucasian sisters
3,4 = Asian siblings
5,6 = Unrelated Asians

Grey shading = the different SNP haplotypes
Boxed area = the overlapping region

Zelinski *et al*., *Nat. Genet.* 2012;44,131

Gene Encoding Jr^a Identified

- **The 397,000 region of homology on chromosome 4q contained 4 validated genes:**
  - ***MEPE***
  - ***SPPI***
  - ***PKD2***
  - ***ABCG2***
- **Only the product of *ABCG2* was known to be expressed on RBCs**
- **We designed primers that were used by PCR to amplify the coding exons (2-16) of *ABCG2***
- **Purified products were subjected to Sanger Sequencing**

Zelinski et al., *Nat. Genet.* 2012;44,131

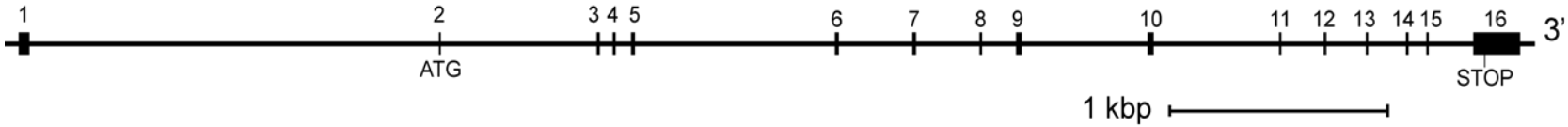
Results of Sequencing the Six Jr(a–) Subjects

Sample	Nucleotide	Exon	Amino acid
Caucasian sisters	c.736C>T homozygote novel	7	Arg246STOP
Asian sibs & unrelated Asian	c.376C>T homozygote	4	Gln126STOP
Asian	c.34G>A homozygote c.244insC heterozygote c.706C/T heterozygote	2 3 7	Val12Met Thr82HisSTOP38 Arg236STOP

Concordant serological, and genetic results established the Jr(a–) blood group phenotype is defined by *ABCG2* null alleles

Gene Encoding JR Glycoprotein: Summary

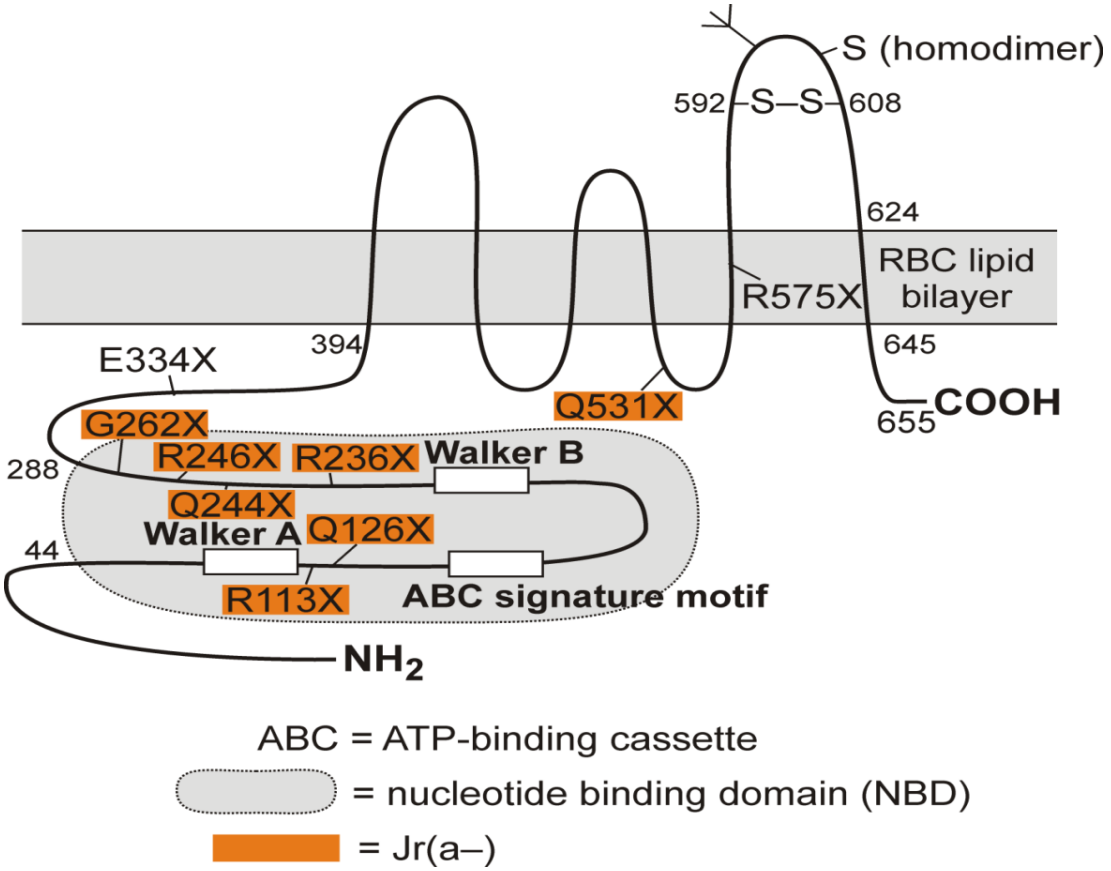
- *ABCG2 (JR)*
- Chromosome 4q22.1
- 16 exons spread over ~68.6 kbp of gDNA



- Nearly 1,300 SNPs (June 2012)
- Highly conserved across species

ABCG2 Membrane Glycoprotein

- **ATP-binding cassette (ABC), sub-family G, member 2 (ABCG2)**
- **breast cancer resistance protein (BCRP)**
- **CD338**



ABCG2 Encoding The Jr(a–) Phenotype

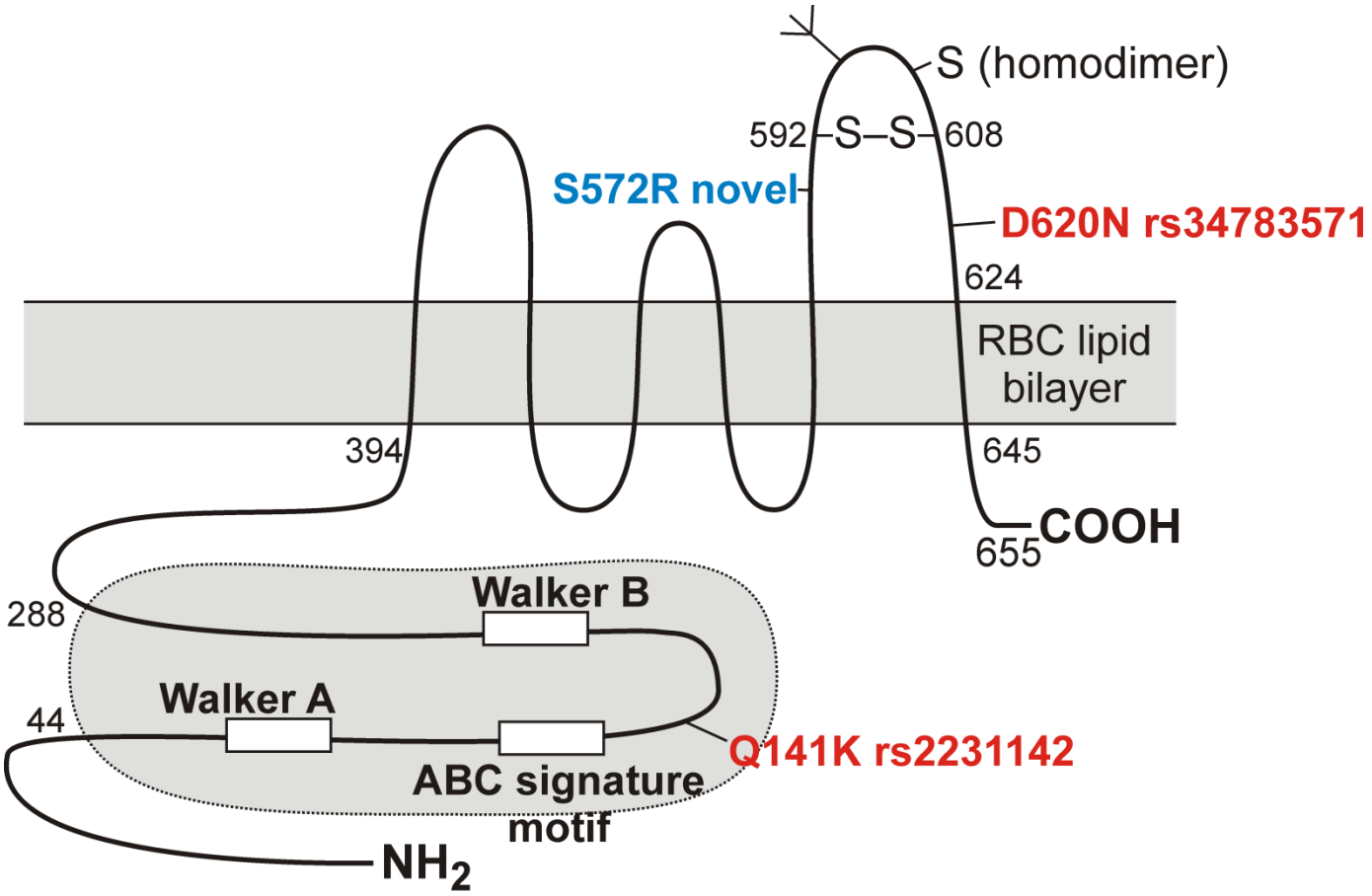
Allele type	Number
Nonsense nucleotide change	**8**
Nucleotide insertion → Frameshift	**3**
Nucleotide deletion → Frameshift	**4**
Total (October 2012)	**15**

Zelinski *et al*., *Nat. Genet.* 2012;44:131

Saison et al., *Nat Genet* 2012;44:174

Hue-Roye, *et al*., *Transfusion* 2012;52:

ABCG2 Encoding the Jr(a+^{W}) Phenotype



ABC = ATP-binding cassette

= nucleotide binding domain

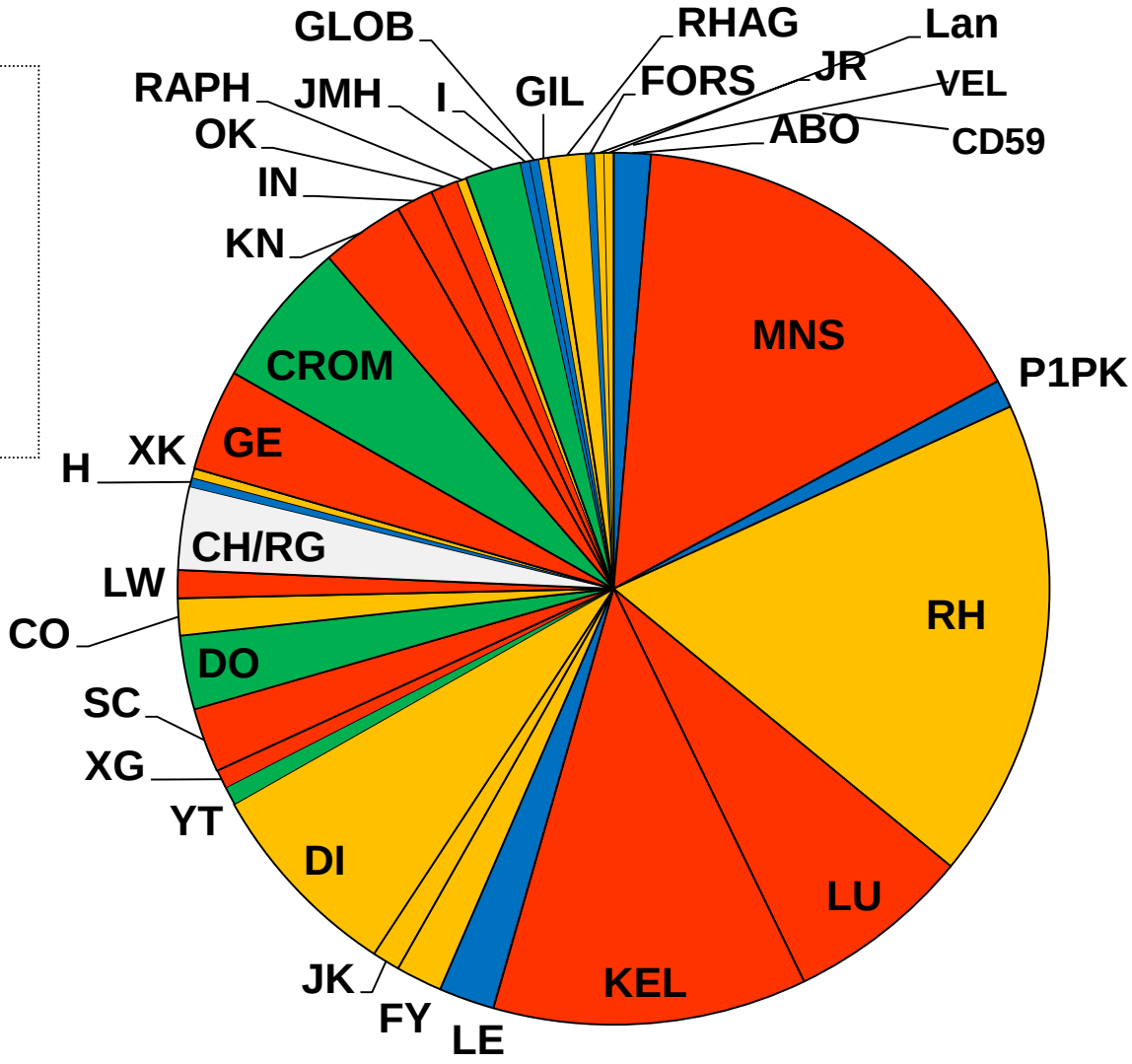
Structure predicted based on information at www.proteinatlas.org

JR Blood Group System (ISBT 032)

- **One antigen – Jr^a (ISBT 032001)**
- **ABCG2 is a member of an ATP-dependent efflux transporter super-family**
- **Wide tissue distribution & broad substrate specificity**
- ***ABCG2* and ABCG2 are the subject of over 2,000 reports (June 2012). By revealing the connection between the Jr(a–) phenotype and ABCG2 immediately provided a wealth of information about the JR blood group system.**
- **Jr(a–) individuals provide a large, cohort (natural knockout) in which to study the exact role and function of ABCG2 in normal physiology and pathologic conditions such as cancer**

Blood Groups Systems July 2014

Systems	35
Gene loci	39
Antigens	297
Alleles	~2,000



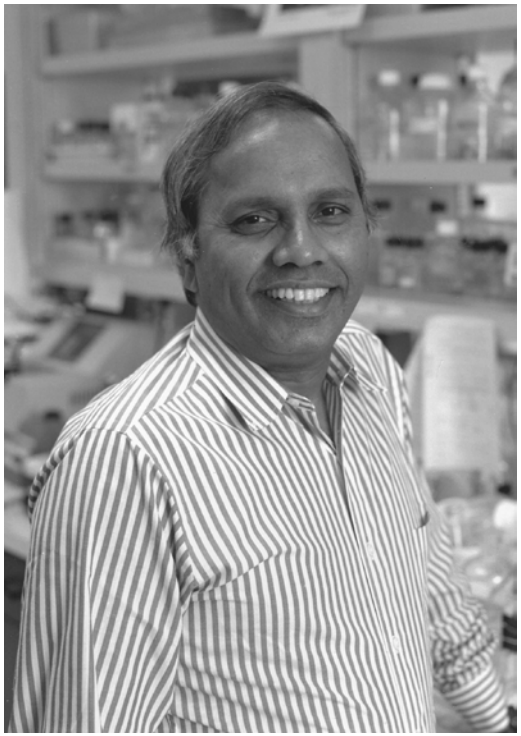
Plus 39 antigens numbered by ISBT

- 15 in 6 Collections
- 17 in 700 Series
- 7 in 901 Series

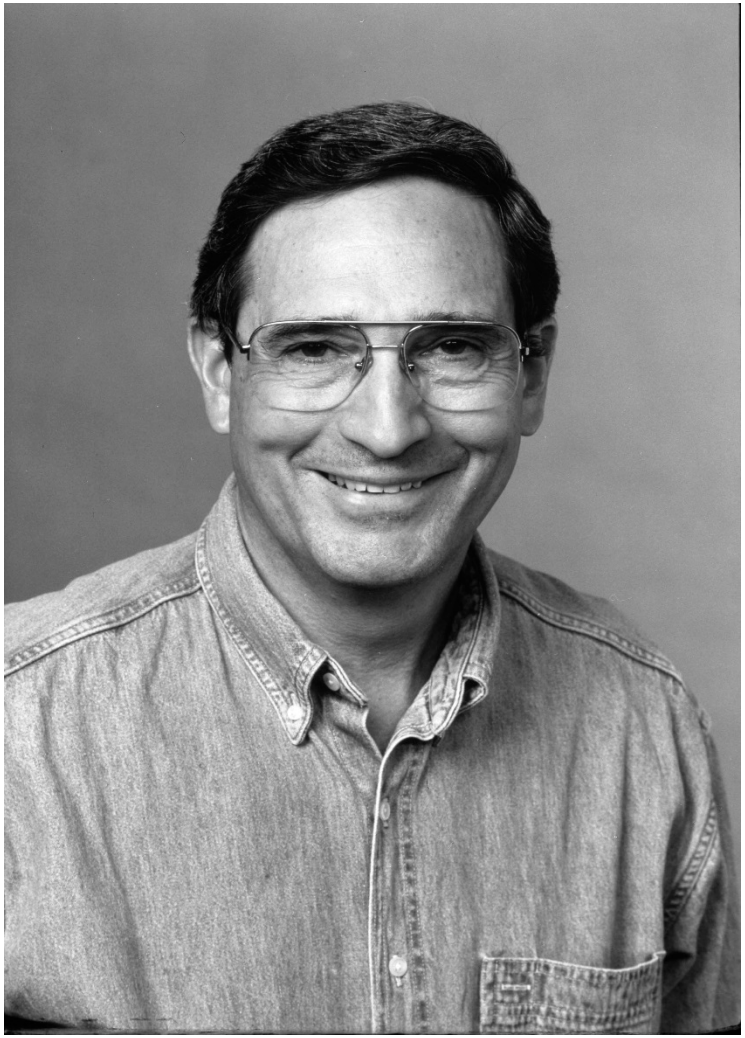
Additional Acknowledgments

Pearl Toy

Dave Anstee and Narla Mohandas



Colvin Redman



Christine Lomas-Francis

Immunohematology Staff (2010)

Immunochemistry Staff (2010)

and to ALL of you …….