INSTITUT NATIONAL DE LA TRANSFUSION SANGUINE

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Donor Genotyping in Practice: Rh Variants and Extended Matching

British Blood Transfusion Society

BBTS ANNUAL CONFERENCE GLASGOW 2017

I declare no conflict of interest related to this presentation

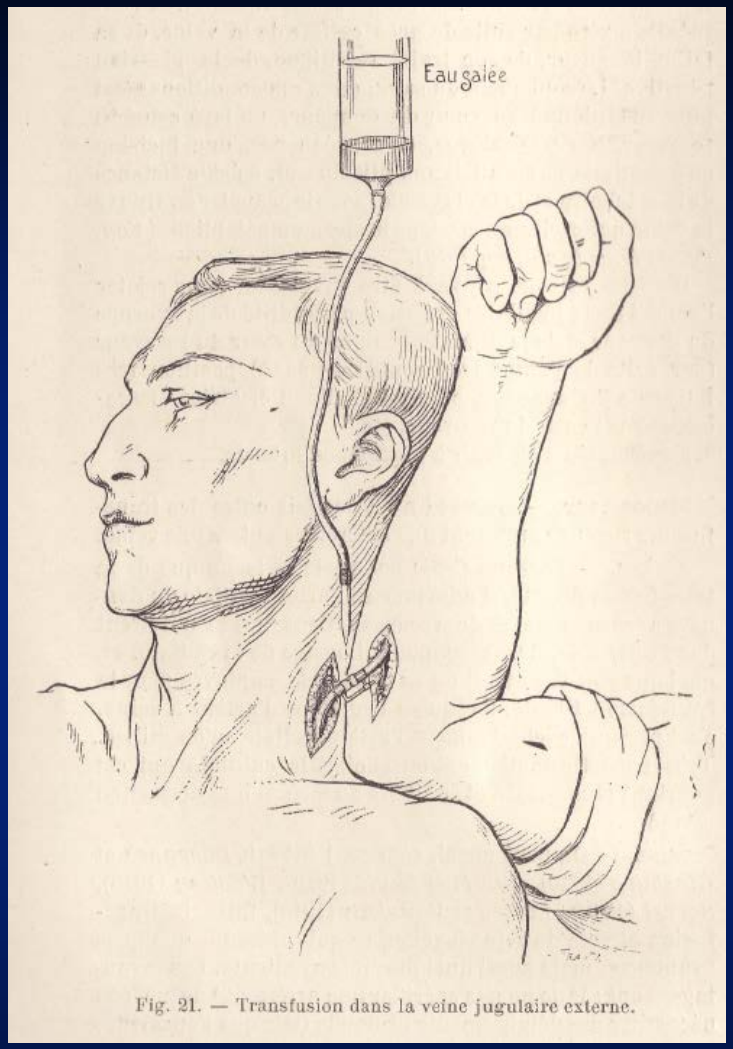
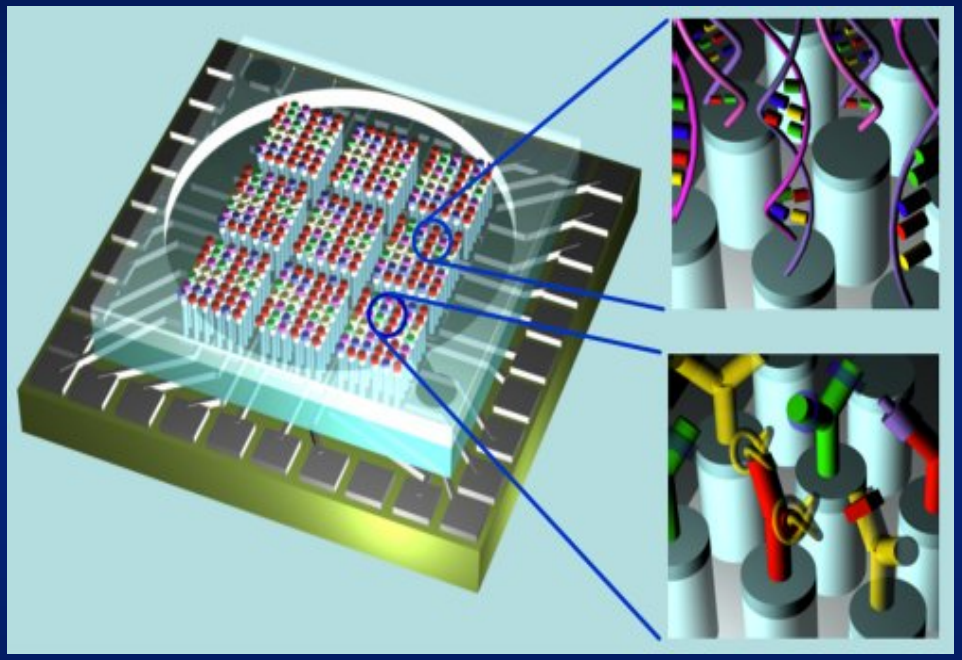
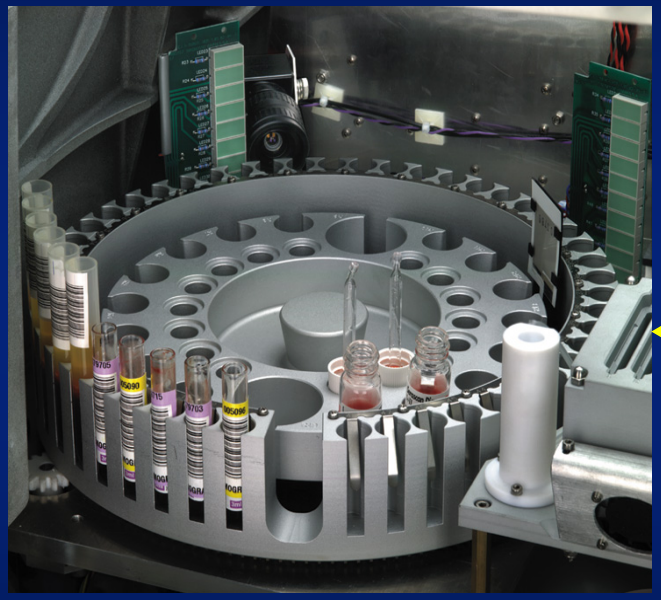


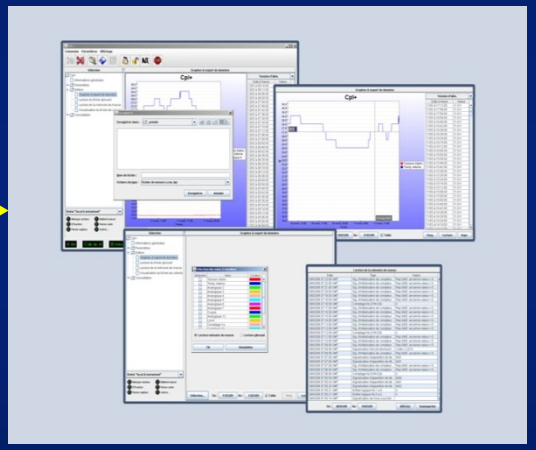
Fig. 21. — Transfusion dans la veine jugulaire externe.

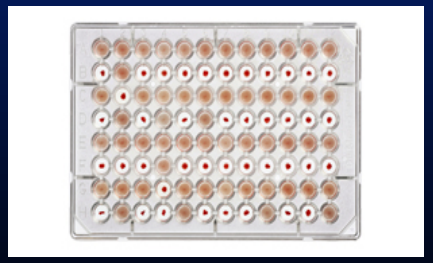


One century!

SEROLOGICAL TESTING







Automation

Workflow +++

Information technology

Full data traceability

2005 – 2008: HIGH EXPANSION OF RBC MOLECULAR TESTING

Bugert P, et al. Microarray-based genotyping for blood groups: comparison of gene array and 5'-nuclease assay techniques with human platelet antigen as a model. *Transfusion* 2005;45:654–659

Denomme GA, Van Oene M. High-throughput multiplex single nucleotide polymorphism analysis for red cell and platelet antigen genotypes. *Transfusion* 2005; 45:660–666

Beiboer SH, et al. Rapid genotyping of blood group antigens by multiplex polymerase chain reaction and DNA microarray hybridization. *Transfusion* 2005;45:667–679

Hashmi G, et al. A flexible array format for large-scale, rapid blood group DNA typing. *Transfusion* 2005; 45:680–688

First CE-marked genotyping platforms from 2008

Transfusion Medicine and Hemotherapy

Review Article · Übersichtsarbeit

Transfus Med Hemother 2009;36:162–167
DOI: 10.1159/000218192

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Accepted: May 5, 2009
Published online: May 28, 2009

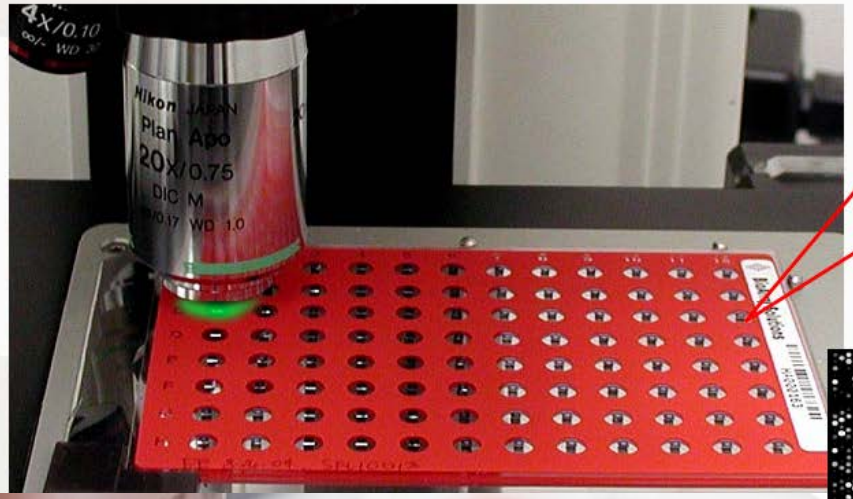
The Bloodgen Project of the European Union, 2003–2009

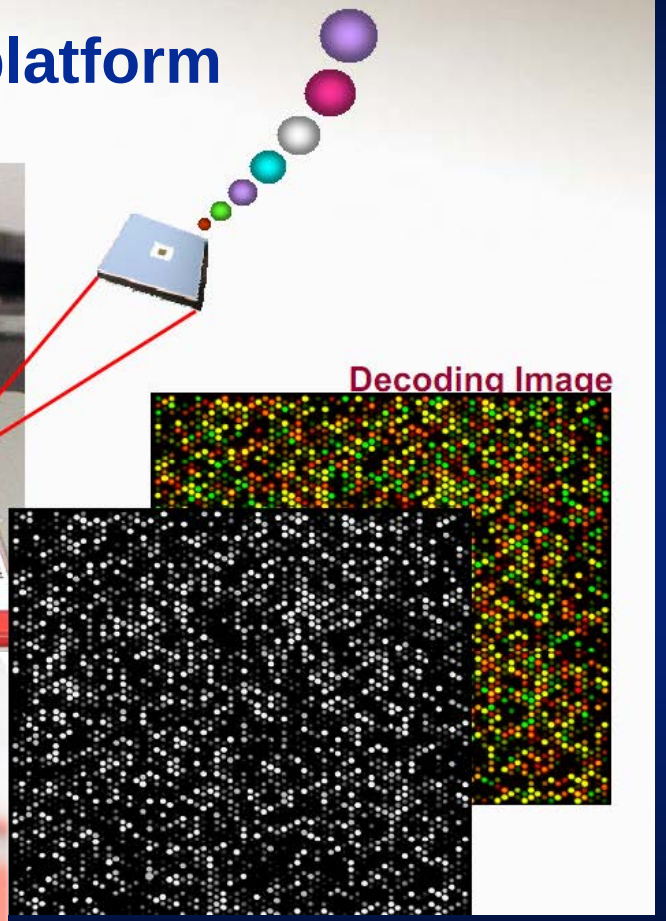
Neil D. Avent[a] Antonio Martinez[b] Willy A. Flegel[c] Martin L. Olsson[d] Marion L. Scott[e]
Núria Nogués[f] Martin Písačka[g] Geoff L. Daniels[e] Eduardo Muñiz-Diaz[f] Tracey E. Madgett[a]
Jill R. Storry[d] Sigrid Beiboer[h] Petra M. Maaskant-van Wijk[h] Inge von Zabern[c]
Elisa Jiménez[b] Diego Tejedor[b] Mónica López[b] Emma Camacho[f] Goedele Cheroutre[h]
Anita Hacker[c] Pavel Jinoch[g] Irena Svobodova[g] Ellen van der Schoot[h] Masja de Haas[h]

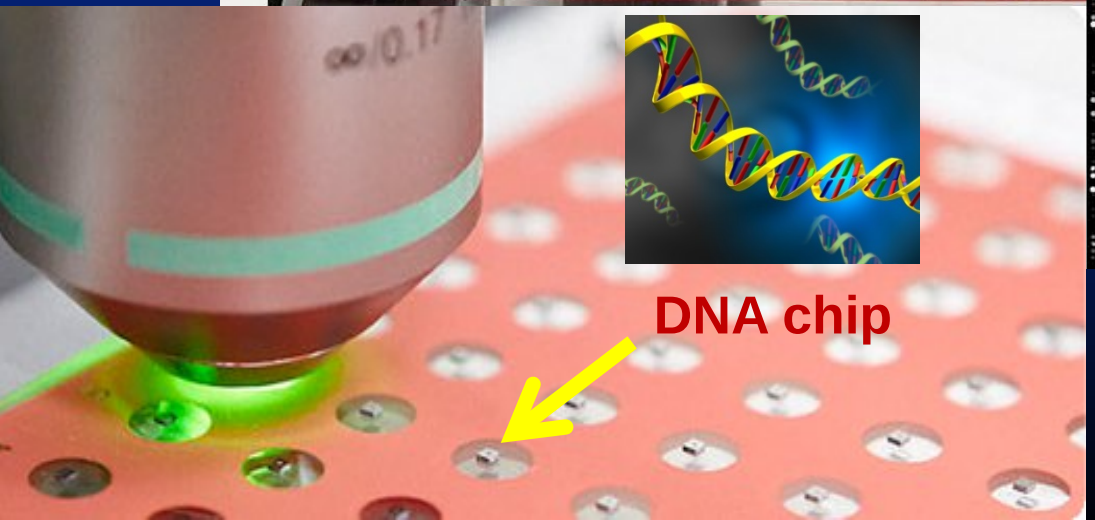
This project had led to the development of a DNA chip device (glass-array), intended to be used to easily genotype an individual for most clinically significant blood groups

RBC GENOTYPING PLATFORMS

Example of a genotyping platform







DNA chip

Blood typing without the need for RBCs!

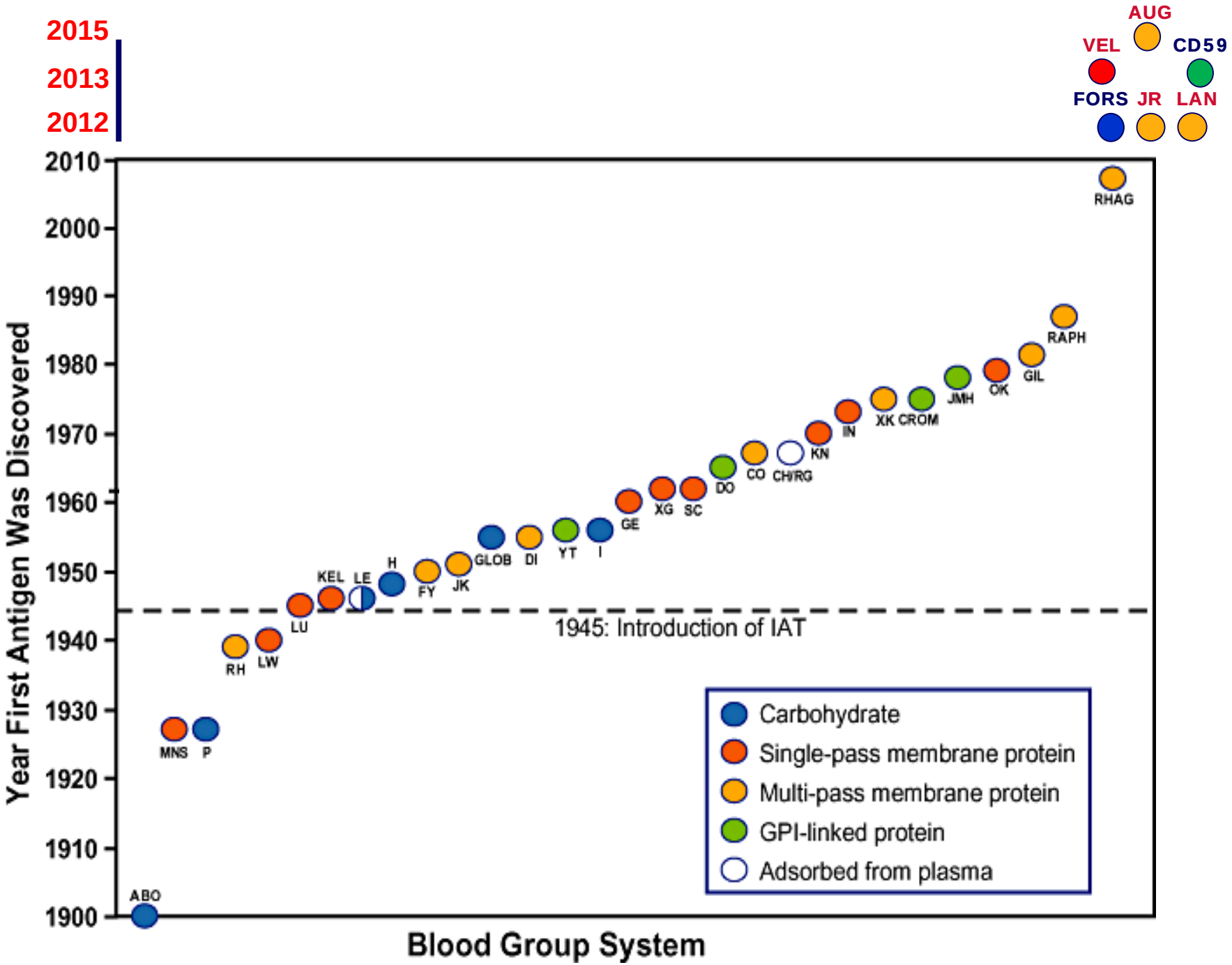
PHENOTYPING
VERSUS
GENOTYPING

SEROLOGY *VERSUS* GENOTYPING

- **Hemagglutination techniques have been considered the gold standard for RBC typing for a very long time**
- **However, several limitations exist for serological typing, which may be overcome by molecular testing**

WHY IS IT POSSIBLE TO PERFORM MOLECULAR TESTING?

- 356 human RBC antigens
- 36 blood group systems
- 40 blood group genes
- Molecular bases of most antigens and phenotypes are known => possible to predict RBC type from DNA study



Adapted from Daniels G & Reid ME. Blood groups: the past 50 years. *Transfusion*. 2010;50:281-9

WHY PERFORMING MOLECULAR TESTING IN BLOOD DONORS?

REAGENTS IN SHORT SUPPLY OR OF POOR QUALITY

- **Short supply**
  - Antibodies to high-prevalence antigens: anti-U, anti-Js^b, anti-Jo^a…
  - Antibodies to low-prevalence antigens: anti-VS, anti-Js^a, anti-Wr^a…
- **Poor quality:** anti-Do^a, anti-Do^b, anti-Kn^a, …

⇨ **Molecular testing very helpful in such a background**

WEAKLY EXPRESSED ANTIGENS ROUTINELY UNDETECTABLE

- Typing reagents may not detect weakly expressed RBC antigens in blood donors, potentially responsible for alloimmunization in the blood recipient
- Typical example is the **DEL type** (very weak expression of D, especially found in Asians)
- ~ 0.1 – 0.3% of serologically D- Caucasians carry an altered *RHD* gene coding for a very low D expression, most being C+ or E+

VERY WEAK D EXPRESSION

Molecular typing in all apparently D- blood donors to avoid D alloimmunization in D negative recipients?

Transfusion and Apheresis Science 50 (2014) 169–174

Contents lists available at ScienceDirect

Transfusion and Apheresis Science

journal homepage: www.elsevier.com/locate/transci

Review

Implementation of a mandatory donor *RHD* screening in Switzerland

Sofia Lejon Crottet[a,1], Christine Henny[a,1], Stefan Meyer[b,1], Franziska Still[a], Martin Stolz[a], Jochen Gottschalk[b], Kathrin Neuenschwander[b], Behrouz Mansouri Taleghani[c], Peter Gowland[a], Beat M. Frey[b], Stefano Fontana[a], Hein Hustinx[a], Christoph Niederhauser[a,*,1], Christoph Gassner[b,*,1]

0.15% of apparent D- donors redefined as D+ (all C+ or E+)

Is this a cost-efficient approach? Only in C+ or E+ donors?

Not performed in France: serological “weak D test” in all D-C+ or D-E+ donors

INVESTIGATION OF VARIANT ANTIGENS

Rh variants

- Weak expression of D and RhCE antigens
- Discrepant results between two typing reagents

⇨ Major relevance in donors of African descent, because this may be a **surrogate marker of the presence of a rare Rh blood type**, which could be of great interest for transfusion of sickle cell disease (SCD) patients

EXAMPLE OF THE DAR TYPE

RHD*DAR ***RHCE*ceAR***

Partial D called DAR

Weak D expression with most reagents

Gene conversion

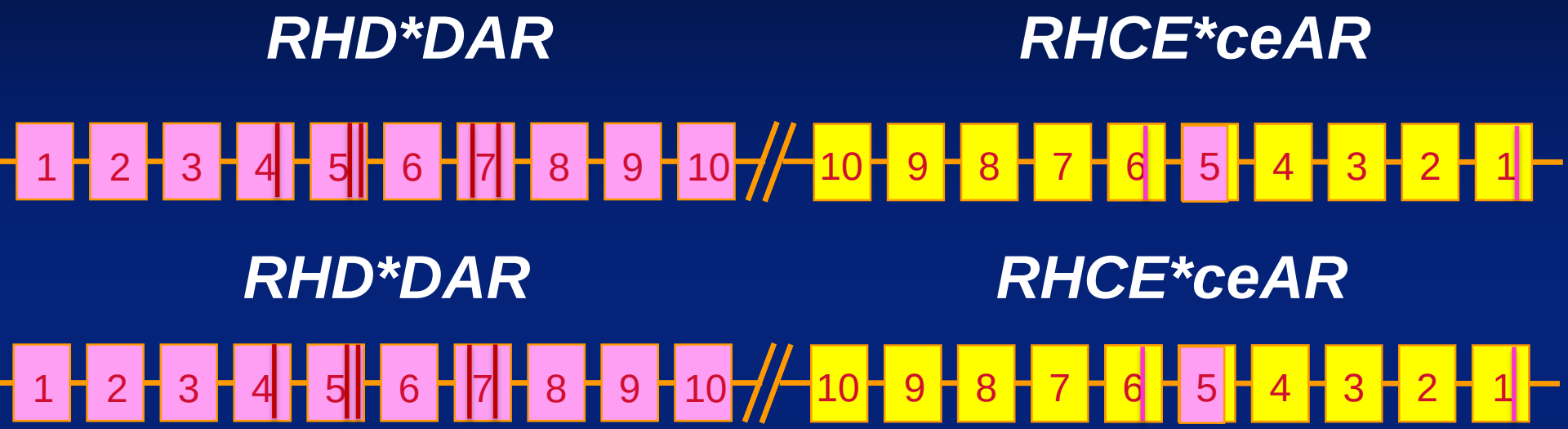
~ 90% of *RHCE*ceAR* alleles are in *cis* to a *RHD*DAR* allele

Hemker MB & al. *Blood* 1999

"AR" in D<u>AR</u> and *ce<u>AR</u>* stands for <u>A</u>msterdam <u>R</u>otterdam

(personal communication with Ellen van der Schoot, Oct 2016)

EXAMPLE OF THE DAR TYPE



If homozygous haplotypes => rare Hr^{S}- blood type (RH:-18)

No weakened c and e with routine reagents

=> The only possibility to serologically screen the rare Hr^{S}- type is a weakened D expression => Any weak D reactivity should be investigated in donors of African descent!

MASS SCREENING FOR RARE DONORS

- By definition, rare types need rare antisera to be screened!
- Current genotyping devices are able to simultaneously screen for many rare blood types
- Molecular testing is extremely helpful in order to mass-screen and recruit new rare blood donors

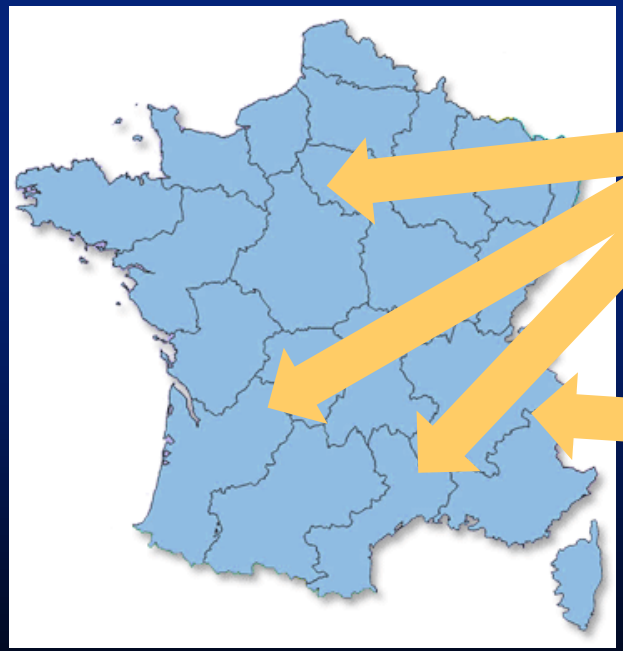
THE FRENCH EXPERIENCE

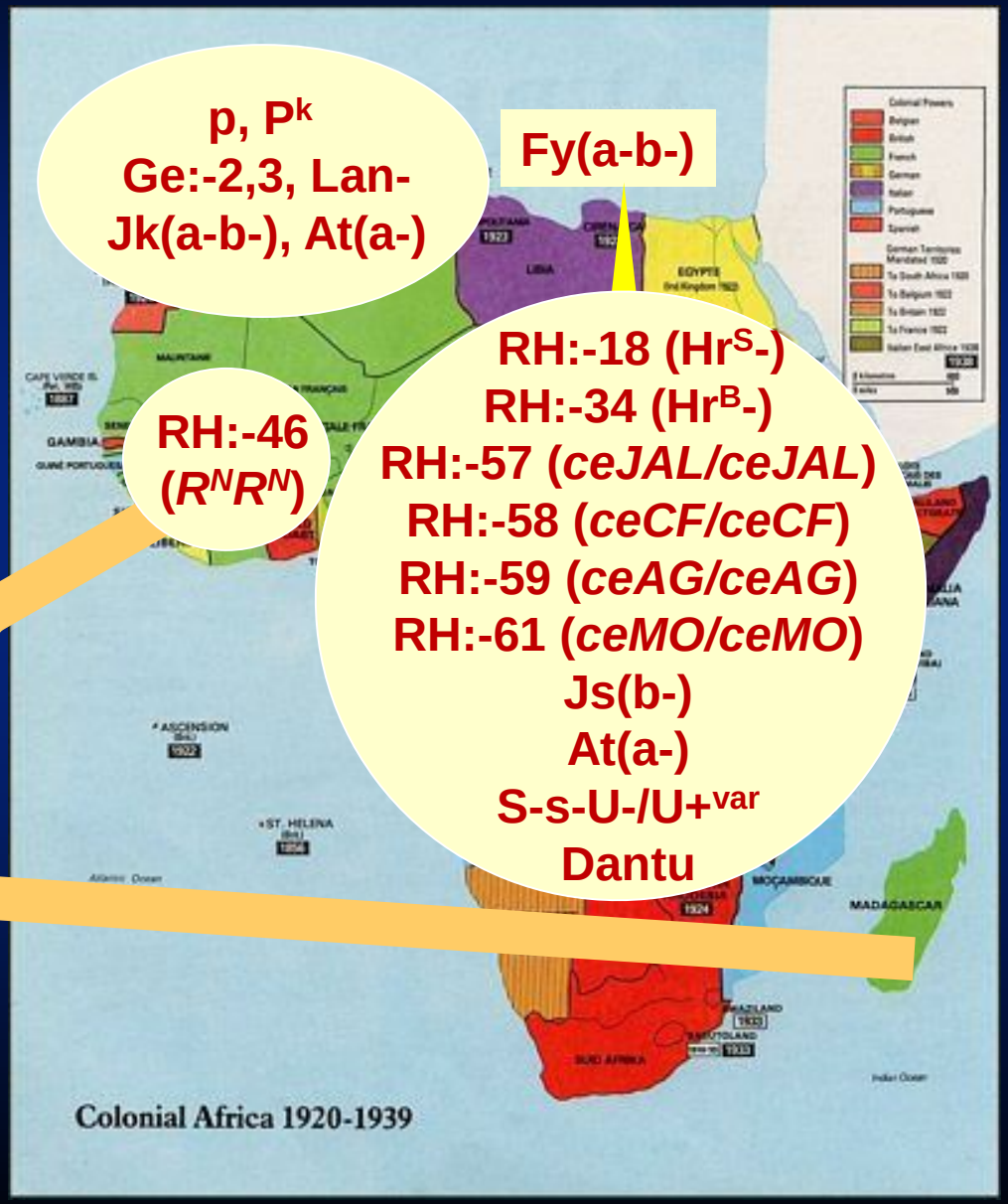
ETHNIC POPULATION BACKGROUND IN FRANCE

- **France: 66 million inhabitants**
- **Ethnically mixed population**
  - **Migrant people from the former French colonies of Africa (1st or 2nd generation)**
  - **Overseas territories**
  - **Recent population movements through Southern Europe**

POPULATION BACKGROUND

Former French colonies of Africa





MAJOR TRANSFUSION ISSUES IN FRANCE

- Patients of African descent with a rare blood type and who need chronic transfusion => sickle cell disease patients
- Fetus, newborn and their mother of African descent, alloimmunized to a high-prevalence antigen
- Emergence of new challenges due to recent population movements through Southern Europe

SICKLE CELL DISEASE IN FRANCE

- **Sickle cell disease (SCD) is the most frequent genetic disease in France (~15,000 patients, number expected to double by the next 15 years)**
- **Patients often transfused and at high risk of developing RBC alloantibodies => systematic match for Rh (C,E,c,e) and K for over 30 years (1985)**
- **Many rare blood types in people of African descent (Rh, MNS, Kell, Dombrock, etc.)**

RH VARIANTS IN PEOPLE OF AFRICAN DESCENT

Significant prevalence of Rh antigen variants of clinical relevance

In our experience (SCD patients)

- ~10% of D+ are partial D
- ~35% of C+ are partial C!
- ~3-5% of e+ are partial e
- ~2% of c+ are partial c

Usually not well screened by standard typing reagents

RH VARIANTS IN AFRICANS

Essential to distinguish common *RHD/RHCE* variants and those with a proven clinical significance (peer-reviewed publications). No international consensus…

In France, for example:

- **DAU-0 is not considered being a partial D**
- ***RHCE*ce48C,733G* is very common in Africans, but not considered at risk. Allele usually claimed to encode a rare hr^B- type (partial e) but no proven alloanti-hr^B in homozygous patients. In USA and Brazil, considered a rare type and need for the same genotype in case of transfusion**

GENOTYPING IN SCD PATIENTS

- **New policy implemented in late 2016 :**
  All SCD patients are subject to *RHD* and *RHCE* genotyping, except those with ≥ 12 transfusion episodes being non-alloimmunized (including at least one transfusion with D+ RBCs in R_0R_0 patients) (considered "low-responders")

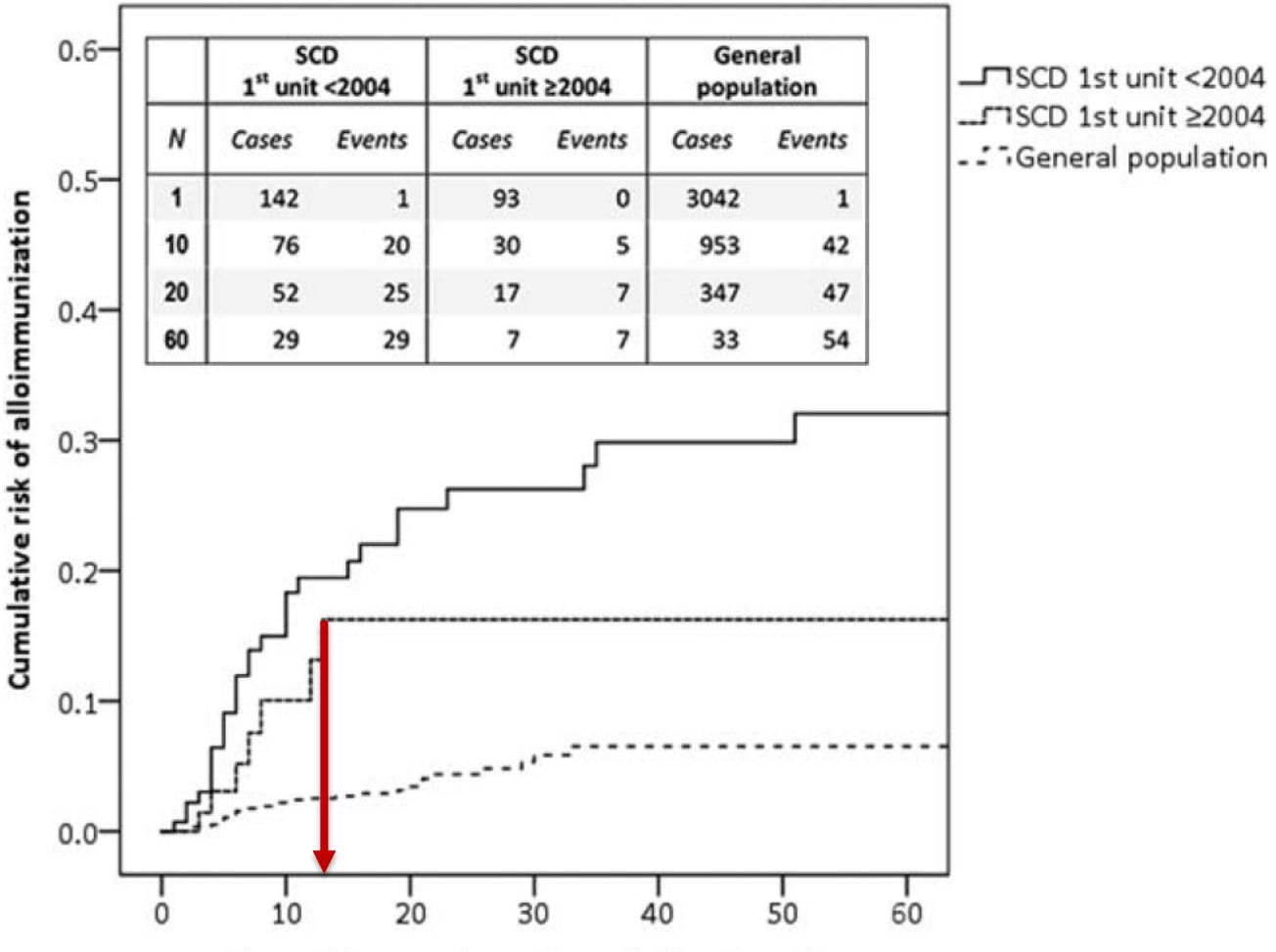
  => "cost/efficiency" approach (genotyping not reimbursed so far!)
- **This threshold value of 12 RBC units is consistent with a work published by a Dutch team in August 2016**

Early occurrence of red blood cell alloimmunization in patients with sickle cell disease

Joep W.R. Sins,[1,2] Bart J. Biemond,[2] Sil M. van den Bersselaar,[1,2] H. Heijboer,[1] Anita W. Rijneveld,[3] Marjon H. Cnossen,[4] Jean-Louis H. Kerkhoffs,[5] Alfred H. van Meurs,[6] F.B. von Ronnen,[1,2] Saurabh Zalpuri,[7] Yolanda B. de Rijke,[8] C. Ellen van der Schoot,[9] Masja de Haas,[9] Johanna G. van der Bom,[7] and Karin Fijnvandraat[1,10]*

AJH

	SCD 1st unit <2004		SCD 1st unit ≥2004		General population	
N	*Cases*	*Events*	*Cases*	*Events*	*Cases*	*Events*
1	142	1	93	0	3042	1
10	76	20	30	5	953	42
20	52	25	17	7	347	47
60	29	29	7	7	33	54



Rh/K matched

American Journal of Hematology, Vol. 91, No. 8, August 2016

RHCE DNA chip used in France

*RH*C*	*ceSL*
*RH*E*	*ceTI*
*RH*c*	*ceRT*
*RH*e*	*ceRA*
*RH*C^{w}*	*Ce-D(4)-Ce [R^{N}]*
*RH*C^{x}*	*DHAR*
ceAR	*r^{G}*
ceEK	*CeMA*
ceBI	*CeVA*
ceMO [ce(667)]	*CeVG*
ce^{S} [ce(733,1006]	*E type I*
ce^{s} [ce(733)]	*E type II (EKK)*
ce^{s}(340)	*E type III*
ce^{S}(748)	*E type IV*
ceCF	*EKH*

Example of the RHCE chip: 6 rare Rh blood types simultaneously screened in people of African descent

Hr^{S}- (RH:-18)

Hr^{B}- (RH:-34)

***R^{N}R^{N}* (RH:-46)**

RH:-57
(RHCE*ceJAL/RHCE*ceJAL)

RH:-58
(RHCE*ceCF/RHCE*ceCF)

RH:-61
(RHCE*ceMO/RHCE*ceMO)

*RH*C*	*ceSL*
*RH*E*	*ceTI*
*RH*c*	*ceRT*
*RH*e*	*ceRA*
*RH*C^{w}*	*Ce-D(4)-Ce [R^{N}]*
*RH*C^{x}*	*DHAR*
ceAR	*r^{G}*
ceEK	*CeMA*
ceBI (& *ceSM* screening)	*CeVA*
ceMO	*CeVG*
ce^{S}	*E type I*
ce^{s}	*E type II (EKK)*
ce^{s}(340)	*E type III*
ce^{S}(748)	*E type IV*
ceCF	*EKH*

Two major limitations

c.254C>G mutation (*ceAG*) (RH:-59 if homozygous) and heterozygous *R^{N}* variants are not screened

=> 2 additional tests

- SSP-PCR for *R^{N}* screening in all C+ patients

- SSP-PCR for *RHCE*ce254G* screening

RHCE*ceAG **found at the heterozygous state in 20% (30/150) of a cohort of young SCD patients in France!**

=> allele frequency = 10% (manuscript in preparation)

HOW DO WE FIND DONORS OF INTEREST?

BLOOD DONOR PHENOTYPING

RBC genotyping is not routinely performed in blood donors in France, even in most donors of African descent. Why?

- Rh (C, E, c, e)/K phenotype systematically tested on all donors (since the early 90s)

- Extended phenotype (Fy^a/Fy^b, Jk^a/Jk^b, S/s) available in ~20% of all A/O donors, whatever their origin => still much cheaper than genotyping in France

- Extended phenotype (including M/N) performed in all A/O/B donors of African origin, and R_oR_o donors if ethnic origin not reported

BLOOD DONOR GENOTYPING

- **Repeat Fy(a-b-), O/A/B, C-E-, K-, Jk(b-), S-donors of African descent, tested for their V, VS, Js^a/Js^b and Do^a/Do^b/Hy/Jo^a status**
- **Weakened reactivity of any RHCE antigen**
- **Weakened D reactivity and discovery of a *RHD* variant known to be in *cis* to a *RHCE* variant allele (e.g. *RHD*DAR/RHCE*ceAR*)**
- **r‘r (D-C+E-c+e+) donors of African origin: at risk of carrying the rare Hr^B- type (RH:-34) in ~3% of cases**
- **Investigation of the molecular basis of the rare S-s- type**

THE S-s- RARE BLOOD TYPE

- Mean prevalence in Africans is 1-2% but may reach up to 35% in equatorial Africa
- **Highest gap between supply and demand in France!**
- 8% of the requested rare units within the last 3 years in France
- Also becomes a major problem in several European countries

THE RARE S-s- BLOOD TYPE

- **Several molecular backgrounds in Africans**
  - **~ 50% are S-s-U- => deletion of the *GYPB* gene**
  - **~50% are S-s-U+^{var} => weak and partial U (alteration of *GYPB*)**
    - **P2 (90%)**
    - **NY (10%)**
- **All are able to develop anti-U, with the strongest examples found in S-s-U-**
- **S-s-U+^{var} RBCs are not compatible with anti-U made by S-s-U- patients**

THE S-s- RARE BLOOD TYPE

- **S-s- donors are systematically genotyped in France**
  - –78 U- donors
  - –83 U+^{var} (76 P2, 7 NY)
- **340 patients with alloanti-U reported in France, including 70 patients with severe SCD**

A FEW THOUGHTS ABOUT *RHCE* ALLELE MATCHING IN PATIENTS OF AFRICAN DESCENT

RHCE ALLELE MATCHING

- Allele matching or "dry matching"
- If a *RHCE* genotype is systematically performed in SCD patients, will we have a sufficient number of matching donors?
- Which *RHCE* alleles are considered of clinical relevance => need for more published data and international consensus
- What to do with the frequent compound heterozygous patients and donors, e.g. *RHCE*ceAR/RHCE*ceMO*? => No concrete data available about the possible trans "counterbalancing" of lacking epitopes

RHCE ALLELE MATCHING

- **What is a "perfect match"?!**

ELSEVIER

Contents lists available at ScienceDirect

Transfusion and Apheresis Science

journal homepage: www.elsevier.com/locate/transci

Short Report

The role of molecular typing and perfect match transfusion in sickle cell disease and thalassaemia: An innovative transfusion strategy

Rossana Putzulu*, Nicola Piccirillo, Nicoletta Orlando, Giuseppina Massini, Maddalena Maresca, Fernando Scavone, Bianca Maria Ricerca, Gina Zini

Transfusion Medicine Department, Haematology Institute, Università Cattolica Sacro Cuore, Rome, Italy

- **Concept of "*RHCE genotype matching tiers*" (American Red Cross, USA)**

Tier 1	**Perfect match on both alleles**
Tier 2	**Donor homozygous for one of the alleles of the patient**
Tier 3	**Donor has the same phenotype (e.g. hr^S-) but different allele(s)**

RHCE ALLELE MATCHING

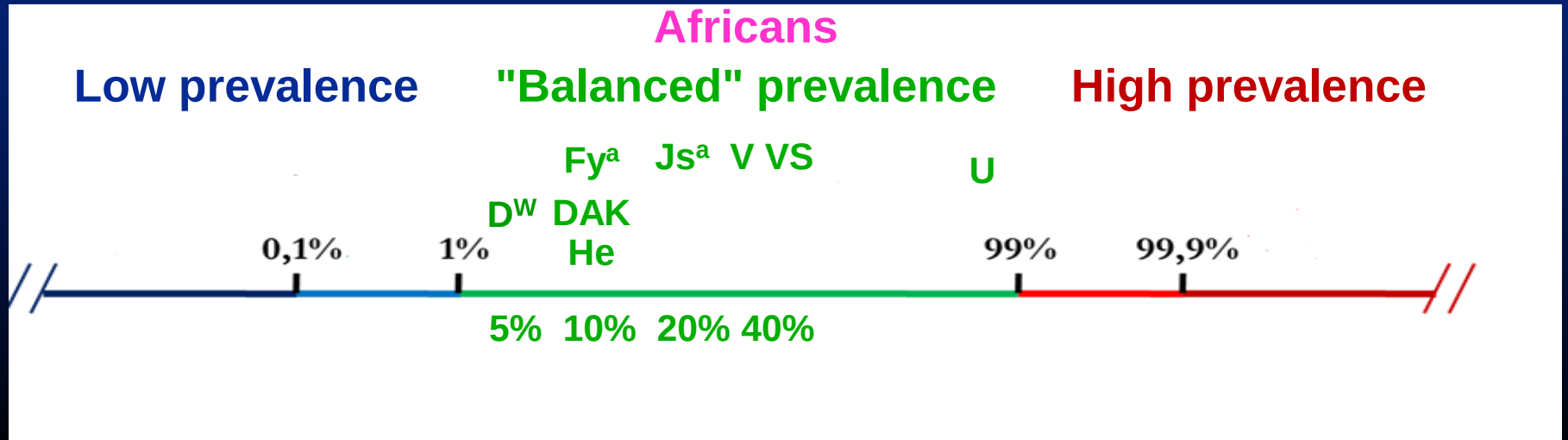
- **Genotype data integration in the laboratory information software. How to do this? >150 *RHCE* alleles and thousands of possible combinations of genotypes!**
- ***RHCE* allele matching logically leads to a systematic intra-ethnic transfusion background, with other risks to be aware of…**

INTRA-ETHNIC TRANSFUSION

- Most Africans are D+C-E-c+e+, Fy(a-b-), Jk(a+b-), S-s+ (if Fy(b-) excluded, compatible blood is present in less than 0.3% of Europeans)
- Some antigens are very rare in Europeans but frequent in Africans (e.g. V, VS, D^W, DAK, Js^a, He, etc.)

INTRA-ETHNIC TRANSFUSION

- **Some antigens are very rare in Europeans but frequent in Africans (e.g. V, VS, D^W, DAK, Js^a, He, etc.)**



INTRA-ETHNIC TRANSFUSION

If RBC units are transfused in an intra-ethnic background, significant risk of developing anti-VS, anti-Js^a, anti-D^W, etc., which are not detectable by routine antibody screening panels! => systematic crossmatch in all SCD patients in France

	Rh-Hr						Kell						Duffy		Kidd		Lewis		P	MNS				Lutheran		Sex-linked
	D	C	E	c	e	C^w	K	k	Kp^a	Kp^b	Js^a	Js^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	P_1	M	N	S	s	Lu^a	Lu^b	Xg^a
I	+	0	0	0	+	0	+	+	0	+	0	+	0	0	0	+	0	0	0	+	+	0	+	0	+	0
II	+	+	+	+	0	0	0	+	0	+	0	+	+	+	+	0	+	+	+	+	0	+	+	0	+	+
III	0	0	0	+	+	0	0	+	0	+	0	+	+	0	0	+	0	+	0	+	+	0	+	0	+	+

⇒ ***RHCE*** **allele matching implementation should require a systematic serological crossmatch (electronic crossmatch not recommended)**

⇒ **Not really a "dry-matching" concept!**

CONCLUSIONS

- Systematic genotype in blood donors not yet performed in France. Only restricted to a subpopulation of repeat donors of African origin
- Cost-efficiency of a large-scale genotyping policy in donors is still a matter of debate
- *RHCE* allele matching in SCD patients is definitely an interesting approach, but leads to new questions and issues
- Availability of new tests should always be considered in a cost-effective way. Could not the extra cost of genotyping be more appropriately used in other aspects of the overall medical care of SCD patients?

THANK YOU FOR YOUR ATTENTION

COST

Integration of red cell genotyping into the blood supply chain: a population-based study

Willy A Flegel, Jerome L Gottschall, Gregory A Denomme

Lancet Haematol 2015; 2: e282–88

Published Online June 3, 2015 http://dx.doi.org/10.1016/S2352-3026(15)00090-3

Summary

Background When problems with compatibility arise, transfusion services often use time-consuming serological tests to identify antigen-negative red cell units for safe transfusion. New methods have made red cell genotyping possible for all clinically relevant blood group antigens. We did mass-scale genotyping of donor blood and provided hospitals with access to a large red cell database to meet the demand for antigen-negative red cell units beyond ABO and Rh blood typing.

Findings We analysed genotype data for 43 066 blood donors. Requests were filled for 5661 (99·8%) of 5672 patient encounters in which antigen-negative red cell units were needed. Red cell genotyping met the demand for antigen-negative blood in 5339 (94·1%) of 5672 patient encounters, and the remaining 333 (5·9%) requests were filled by use of serological data. Using the 42 antigens represented in our red cell genotype database, we were able to fill 14 357 (94·8%) of 15 140 requests for antigen-negative red cell units from hospitals served by the BloodCenter of Wisconsin. In the pilot phase, the seven hospitals identified 71 units from 52 antigen-negative red cell unit requests.

matched red blood cells for transfusion. Flegel and colleagues' study was funded by their blood centre and the Intramural Research Program of the National Institutes of Health. However, they do not provide an estimate for the implementation costs of such a programme. The investigators refer to a study of the financial implications of *RHD* genotyping for clarification of serological weak D laboratory test results.[5] However, I am unaware of any studies that analyse large-scale automated blood group genotyping to assist blood centres that are considering replicating the present study[1] in routine practice. In the USA, reimbursement to hospitals and blood centres for the supply of antigen-negative red blood cell units for patients with alloantibodies is limited to the cost of collection and testing of only the unit of antigen-negative red blood cells that is used. If the donor of that unit, for example, was selected as the one Kp(b–) donor after 1000 units were screened, the cost of tests for the other 999 units is typically not reimbursable in the existing system.

S Gerald Sandler
Department of Laboratory Medicine, MedStar Georgetown University Hospital, Washington, DC 20007, USA

Comment

Blood group genotyping: faster and more reliable identification of rare blood for transfusion

group antigen-negative units. Implementation of mass-scale genotyping for blood donors as a routine procedure is a win-win situation for patients and hospitals. For health-care systems that reimburse blood centres only for the costs of collection and testing of supplied red blood cell units, implementation of this advance must await a creative way to meet the establishment costs that will be paid for by current reimbursement policies.