Donor Genotyping in Practice
Who and How?

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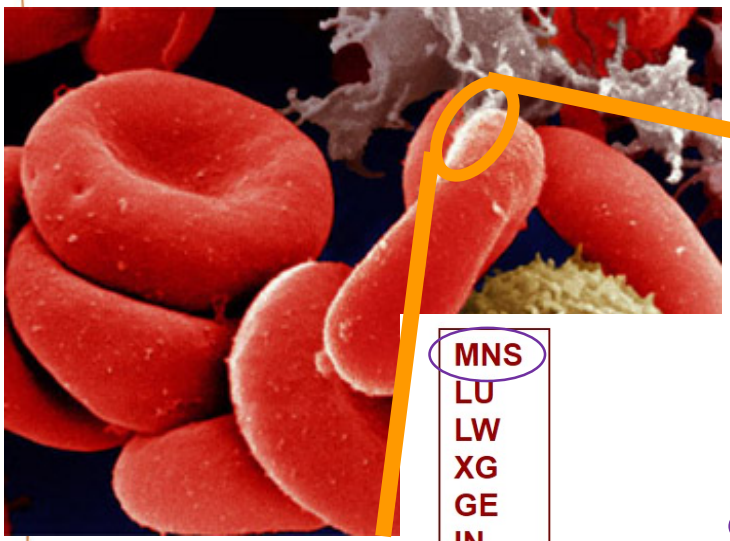
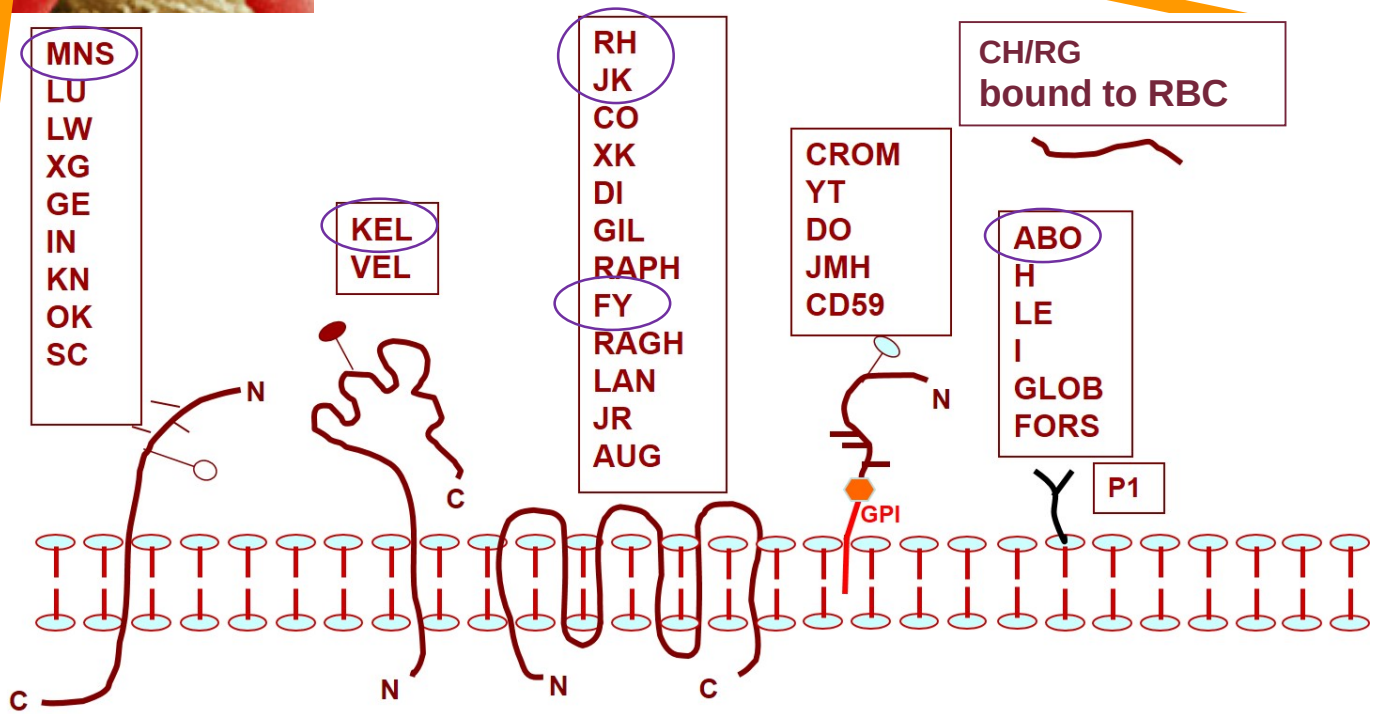
Red cell (glyco)proteins/lipids carry 36 Blood Group systems: 324 antigens





Molecular basis of all Blood Group Systems is known

ISBT	System name	Gene names
1	ABO	ABO
2	MNS	GYPA, GYPB, GYPE
3	P1PK	A4GALT
4	Rh	RHD,RHCE
5	Lutheran	BCAM
6	Kell	KEL
7	Lewis	FUT3
8	Duffy	DARC
9	Kidd	SLC14A1
10	Diego	SLC4A1
11	Yt	ACHE
12	Xg	XG
13	Scianna	ERMAP
14	Dombrock	ART4
15	Colton	AQP1
16	Landsteiner-Wiener	ICAM4
17	Chido/Rodgers	C4A, C4B
18	H	FUT1

ISBT	System name	Gene names
19	Kx	XK
20	Gerbich	GYPC
21	Cromer	CD55
22	Knops	CR1
23	Indian	CD44
24	Ok	BSG
25	Raph	CD151
26	John Milton Hagen	SEMA7A
27	I	GCNT2
28	Globoside	B3GALNT1
29	Gill	AQP3
30	Rh-associated glycoprotein	RHAG
31	Forssman	GBGT1
32	JR	ABCG2
33	LAN	ABCB6
34	VEL	SMIM1
35	CD59	CD59
36	AUG	ENT1

Slide from 2008

The field is moving from serology to DNA typing

EDITORIAL

Goodbye to agglutination and all that?

there is more work to do regarding molecular methods for ABO and D typing before routine application can be contemplated, but in the longer term blood grouping may be swept along with the molecular revolution. Does this

David J. Anstee

Volume 45, May 2005 **TRANSFUSION** 653

Where are we, and where are we going, with DNA-based approaches in immunohematology? Is serology finished?

George Garratty

cber/efoi/minutes.htm). The FDA has shown its interest in DNA-based technologies and is developing its approach to regulating this field. It is obvious that there

the web. Although I believe DNA approaches will one day dominate immunohematology, I left the workshop knowing I would be retired (or dead) before they replace serology in routine immunohematology.

Rh complexities: serology and DNA genotyping

Connie M. Westhoff

Volume 47, July 2007 Supplement **TRANSFUSION**

sion support. With the development of high-throughput platforms, genotyping is poised to move into the mainstream, revolutionizing the provision of antigen-negative donor units. Future implementation of molecular testing for Rh will reduce, and could potentially eliminate, alloimmunization to Rh.

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In 2017:
Has genotyping replaced serology?

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Major theoretical drawbacks of molecular typing

- Presence of gene is NOT always resulting in expression of gene
  - Silent genes due to missense mutations or deletions / inserts resulting in frameshift
  - Mutations in regulatory regions
- Presence of variant genes resulting in loss or gain of expression of epitopes not predicted by standard assays
- Presence of mutations not interfering with expression, but with reliability of genotyping assay

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Remarks on theoretical drawbacks for donor typing

- Mostly resulting in false positive phenotype predictions
  - => more cumbersome for recipient typing than for donor typing
- Most of variants are recurrent (e.g. RHD pseudogene, O-alleles, GATA-FY) and can be easily included in assays
- Most variants resulting in gain of epitopes are also not detected by standard serology
- Genotyping assays can be designed in such a way that they are not hampered by ‘silent’ mutations

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Except for the ABO-system there are no major theoretical drawbacks for donor genotyping

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Practical drawbacks for donor genotyping

- Availability of high throughput assays?
- Pricing:
  - Serology is very cheap
- Conservatism:
  - Serology is very well implemented, and difficult to outcompete

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Availability of commercial genotyping assays

Supplier	Name	Number of blood group systems	Number of antigens (excl variants)	Number of SNPs	Method	Through-put
Progenika (Grifols)	IDCORE XT	10	37	29	Luminex xMAP	16/ 4hrs
Immucor	PreciseType HEA	11	36	24	Bead micro-array	96/ 5 hrs
MRC-Holland	MLPA	18	54	82	MLPA	32/ 24 hrs
Agena Bioscience	Hemo ID™ DQS	3-12	61	7-33	MALDI-TOF MS	3000/8hrs
Life Technologies	Taqman Open Array	variable	42	32	RQ-PCR	96 / 8 hrs
Beckman	GenomeLab SNP stream	6	19-22	11	Single base extension	384/ day?
Applied Biosystems	SNaPshot	1-10	<26	5-39	Minisequencing	Variable (medium)
AXO	HIFI blood 96	9	22	11	microarray	96/ 4.5 hrs
Innotrain	RBC-Ready Gene	1-7	Several panels	<24	SSP-PCR	96/ 3-4 hrs

Practical drawbacks for donor genotyping

- Availability of high throughput assays

- Pricing:
  - Serology is very cheap

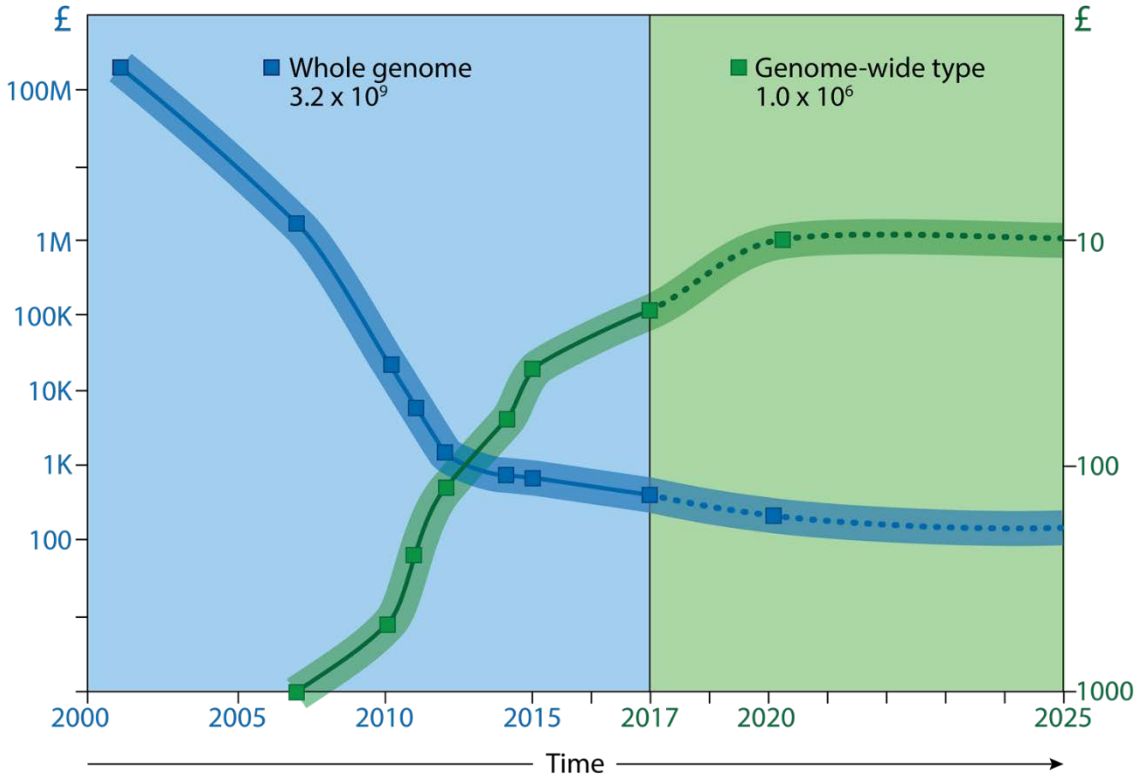
- Conservatism:
  - Serology is very well implemented, and difficult to outcompete

➢ **Genotyping should become better than serology**
  - ➢ More comprehensive
  - ➢ More reliable
  - ➢ Additional advantages
  - ➢ Pricing

Cost of genotyping continues to come down

30x whole genome sequencing

96 samples / run



Genome-wide array
~800,000 snv

384 samples / run

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Large population studies with genotype data

Study	Sample number	NHSBT Blood Typing	Axiom UKBB array	Whole Genome Sequencing
UK Biobank	500,000	✓ (33000)	✓	✗
NIHR BioResource	64,000	✓ (10,000)	✓	✗
INTERVAL RCT	50,000	✓	✓	✓
COMPARE	31,000	✓	✓	✗
100,000 Genomes Project	29,000	✗	✗	✓
NIHR BioResource - Rare Diseases	12,000	✗	✗	✓

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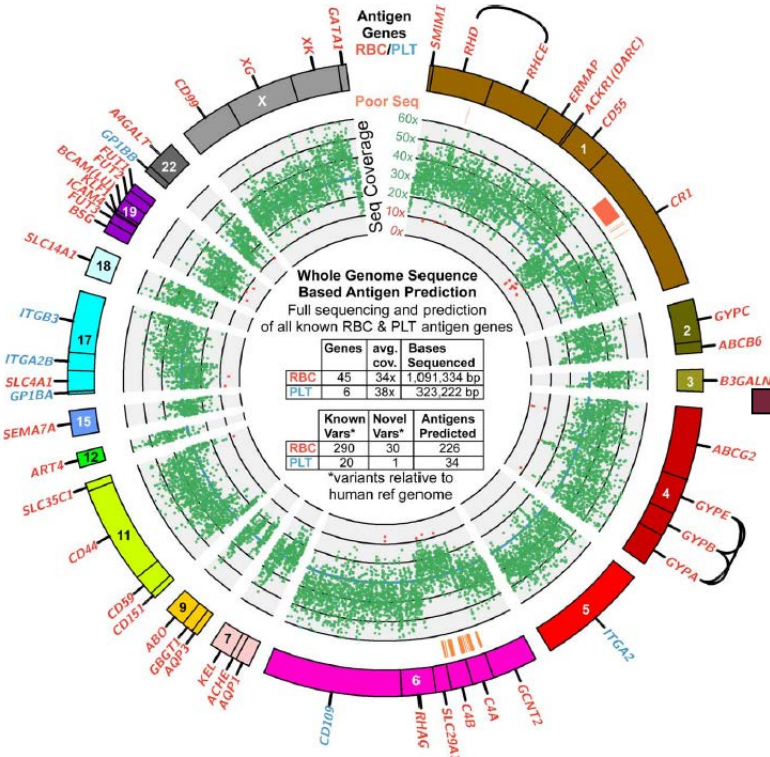
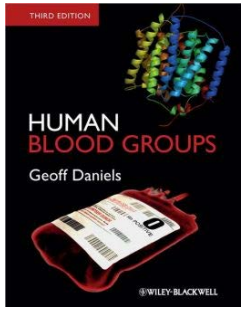
Next Generation Sequencing

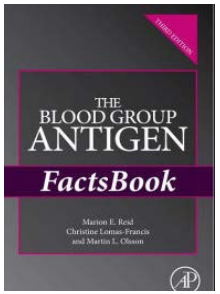
Analysis algoritm



Lane *et al.*, 2016, *Transfusion*

Consolidated Database





ISBT dbRBC

ERYTHROGENE

BloodAntigens.com

Blood Group Report

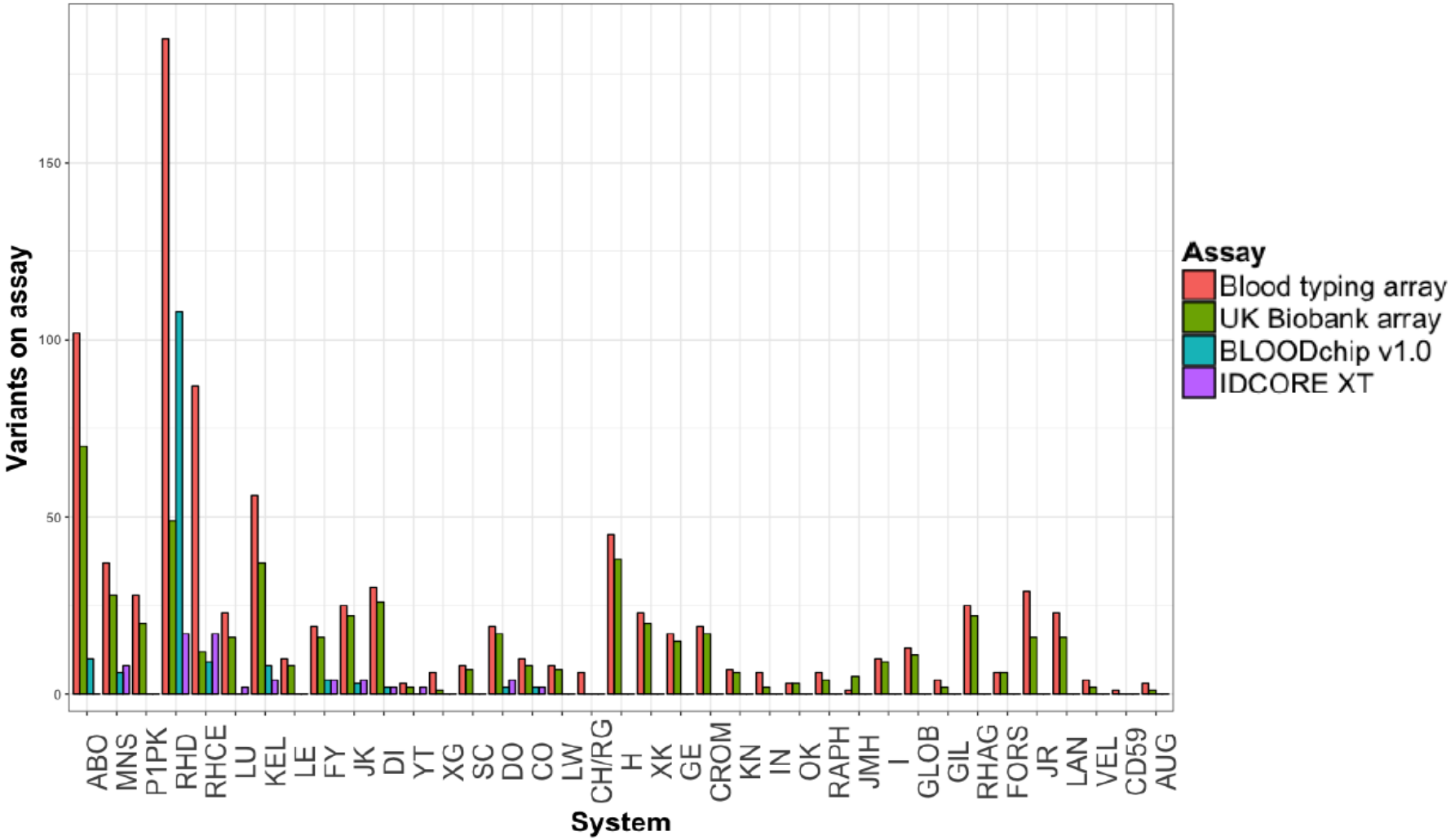
Blood Group Analysis pipeline

UK BioBank Axiom Array (0.8M snvs)

- INTERVAL study : 50.000 donors
- Blood groups retrieved from NHSBT database
- Good results for some antigens
  - S/s – 99.8%
  - C(w) – 100%
  - Lu(b) – 100%
- Some more challenging
  - ABO – 95.4%
  - D - 32.8%
  - M – 27.5%
  - N – 69.6

Blood Group	Individuals Typed
ABO	47,694
Rh D	47,691
Rh C	47,679
RH c	47,686
Rh E	47,686
Rh e	47,681
Rh C(W)	27,682
Kell (K)	47,687
k (k)	5,493
P1	5,493
Jka	26,867
Jkb	26,576
M	26,173
N	3,280
S	24,431
s	17,217
Fya	17,887
Fyb	16,493
Lua	6,243
Lub	10,005
Kpb	9,837
Kpa	6,730
Lea	5,967
Leb	4,387

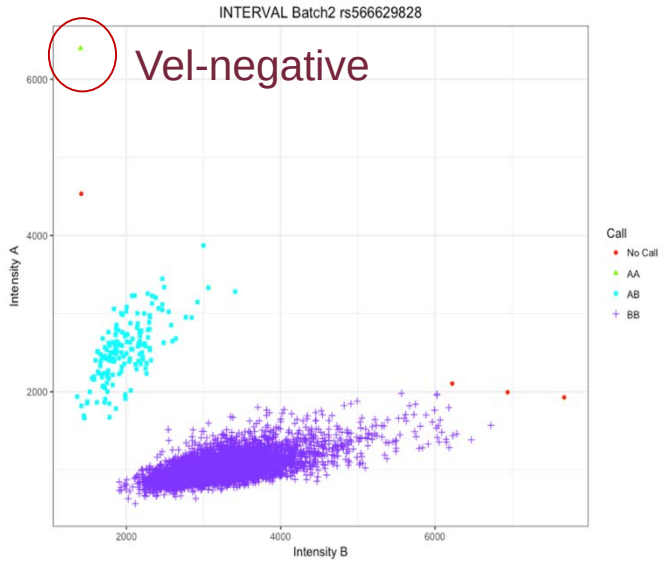
BGC project


Variants on assay
150
100
50
0
ABO
MNS
P1PK
RHD
RHCE
LU
KEL
LE
FY
JK
DI
YT
XG
SC
DO
CO
LW
CH/RG
H
XK
GE
CROM
KN
IN
OK
RAPH
JMH
I
GLOB
GIL
RHAG
FORS
JR
LAN
VEL
CD59
AUG
System
Assay
Blood typing array
UK Biobank array
BLOODchip v1.0
IDCORE XT

Improved performance of BGC v2 Array

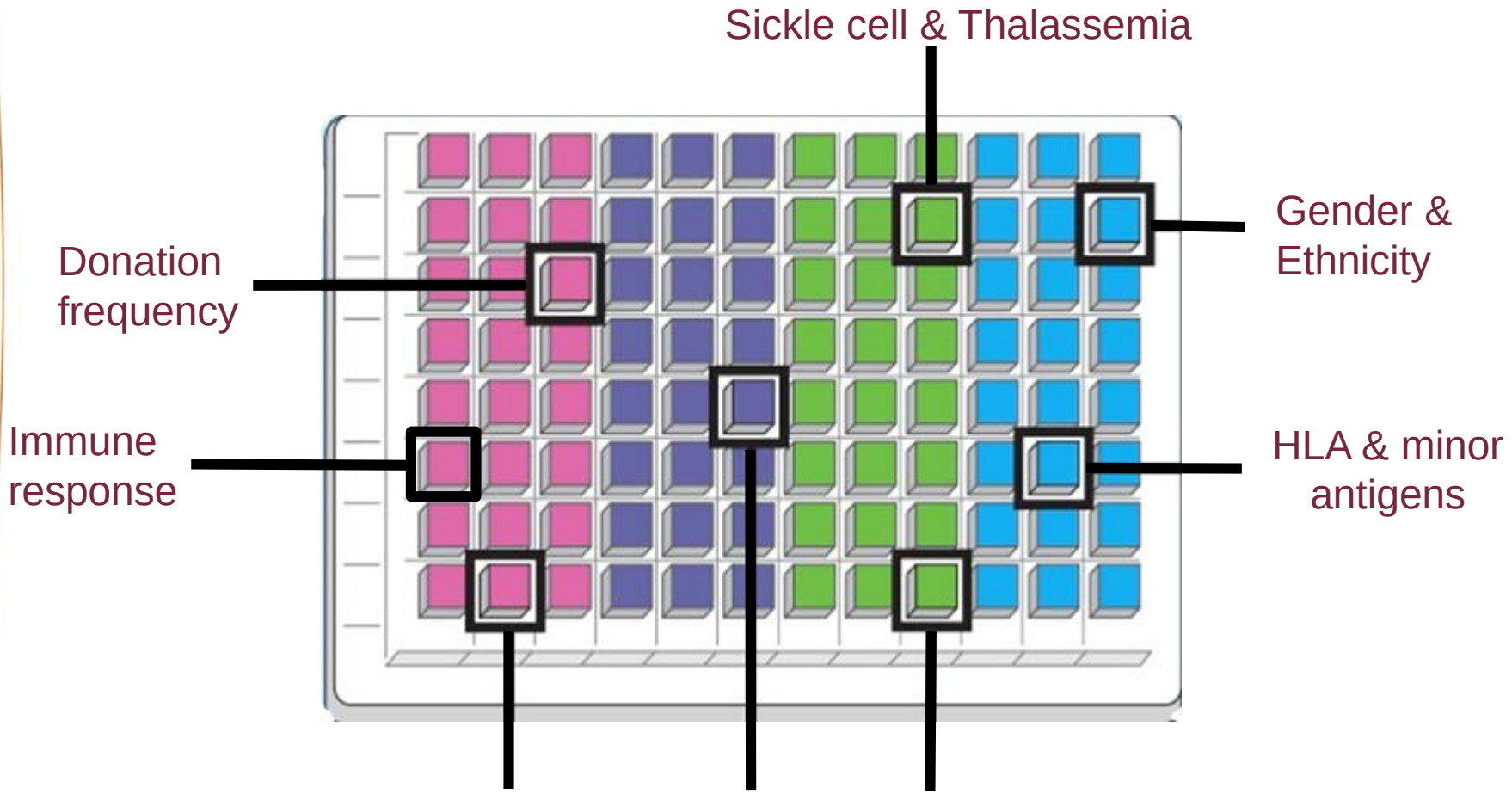
System	Comparisons	Correct	Incorrect	Concordance (%)
ABO	432	430	2	99.5
D	507	506	1	99.8
M	372	371	1	99.7

- Rare donors identified in 50,000 samples:
  - VEL- negative : 3
  - k- (KK) : 74
  - Js(b)-: 6
  - U- 1
  - Di(a)- 1
  - LAN- 4



Gleadall et al.unpublished, preliminary results

SNV array not only for blood group typing



Conclusions (1)

- Integrated platform for RBC grouping, HLA, HPA and HNA typing will be available by 2018

- This platform might allow to get a fully typed blood donor cohort

1) **Identification of rare donors**

2) Preventive matching: 3-5% of all transfusion episodes result in alloimmunisation

   - **Availability of fully matched donors?**

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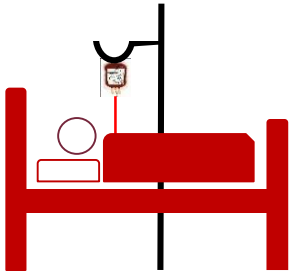
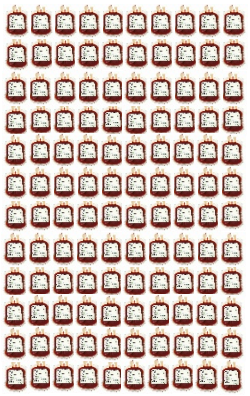


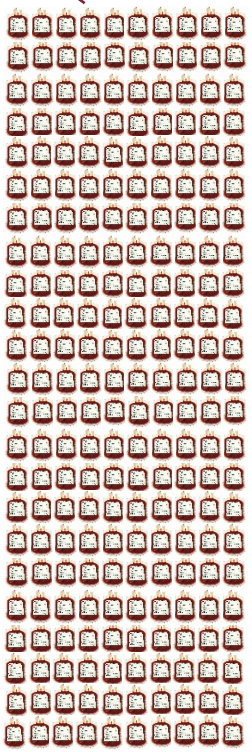
Can a request for a fully matched unit be directly fulfilled from the inventory?

Joost van Sambeeck

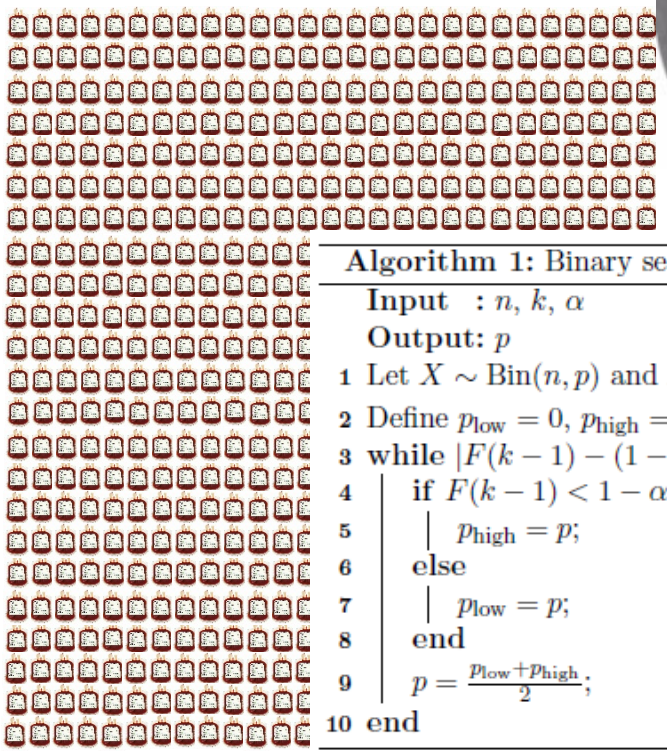
n=60 (small hospital)



n=120 (large hospital)



n=250 (university hosp)



n=1000 (distribution center)

Algorithm 1: Binary search algorithm to find p

Input : n, k, α
Output: p

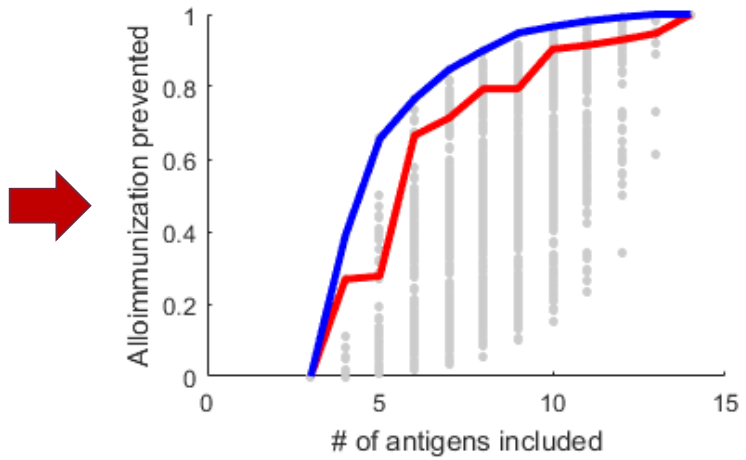
Let $X \sim \text{Bin}(n,p)$ and $F(k) = \mathbb{P}[X \leq k]$;
Define $p_{\text{low}} = 0$, $p_{\text{high}} = 1$, $p = \frac{p_{\text{low}}+p_{\text{high}}}{2}$;
while $|F(k-1) - (1-\alpha)| \geq 10^{-6}$ **do**
  if $F(k-1) < 1-\alpha$ **then**
    $p_{\text{high}} = p$;
  else
    $p_{\text{low}} = p$;
  end
  $p = \frac{p_{\text{low}}+p_{\text{high}}}{2}$;
end

Matching patients for a large number of antigens

- Two matching strategies
  1. **Maximize the percentage of requests that can be fulfilled directly from stock**
  2. **Maximize the percentage of alloimmunisation prevented**

	All (n=21 512)		
anti-C	22 (0·10%)	Anti-Lu^a	31 (0·14%)
anti-c	37 (0·17%)	Anti-Lu^b	0
anti-E	177 (0·82%)	Anti-Le^a	8 (0·04%)
anti-e	4 (0·02%)	Anti-Le^b	3 (0·01%)
anti-K	122 (0·57%)	Anti-M	18 (0·08%)
anti-C^w	19 (0·09%)	Anti-N	1 (0·01%)
anti-Fy^a	24 (0·11%)	Anti-S	8 (0·04%)
Anti-Fy^b	5 (0·02%)	Anti-s	0
Anti-Jk^a	50 (0·23%)	All antibodies	536
Anti-Jk^b	7 (0·03%)	Number of cases	474 (2·20%)

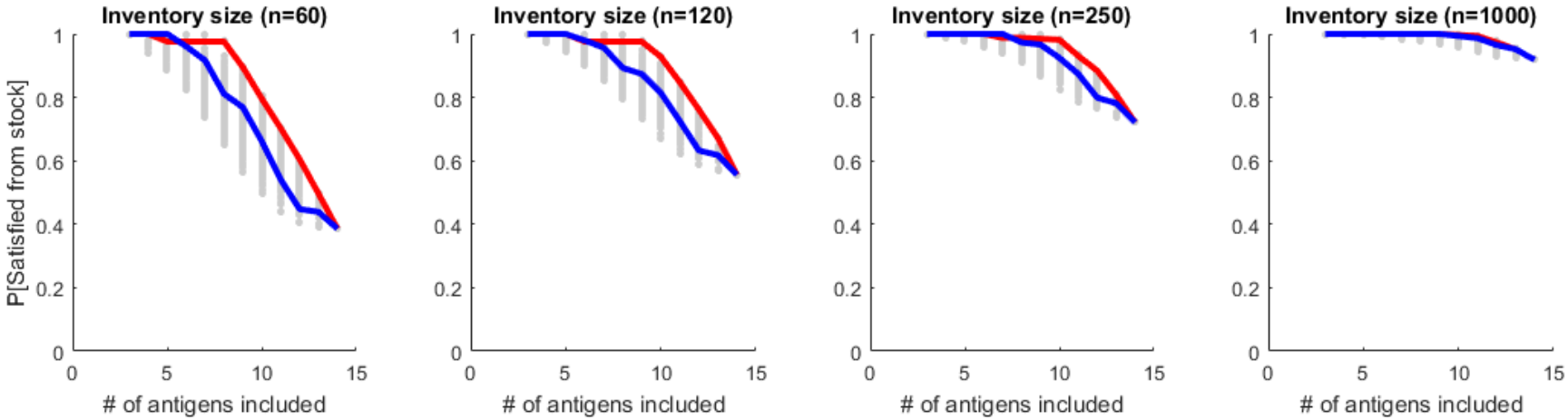
Data are n (%).



Evers et al. Lancet Haematology 2016

Requests fulfilled

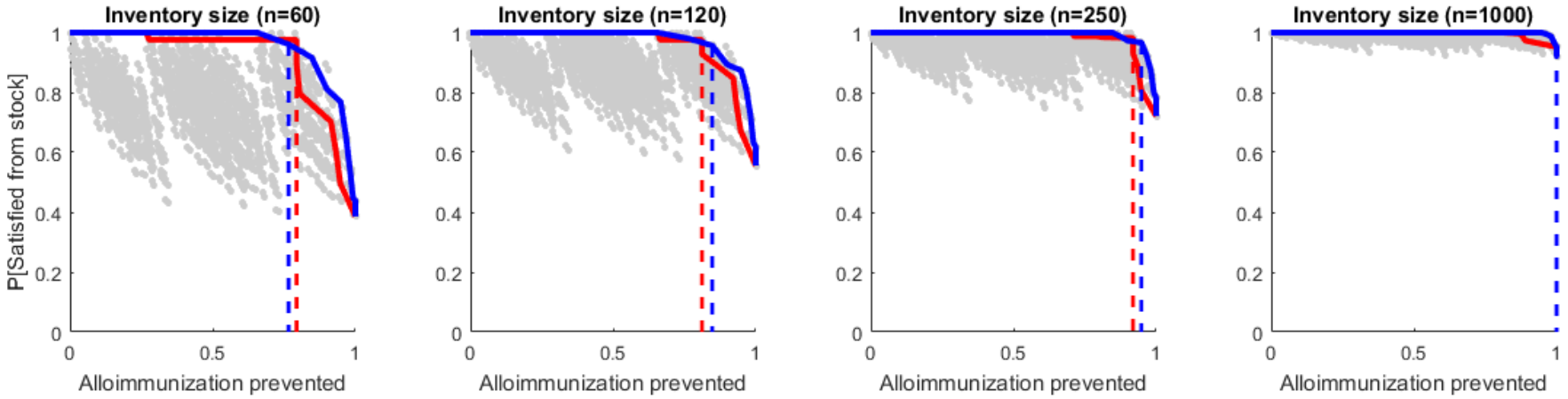
- Patient population **100% European**, donor population **100% European**
- Two matching strategies:
  1. **Maximize the percentage of requests that can be fulfilled directly from stock**
  2. **Maximize the percentage of alloimmunisation prevented**



ABO,D +E+K+Jk(a)+c+Fy(a)+C+S+Jk(b)+Fy(b)+e+s

Alloimmunization prevented / request fulfilled

- Patient population **100% European**, donor population **100% European**
- Two matching strategies
  1. **Maximize the percentage of requests that can be fulfilled directly from stock**
  2. **Maximize the percentage of alloimmunisation prevented**



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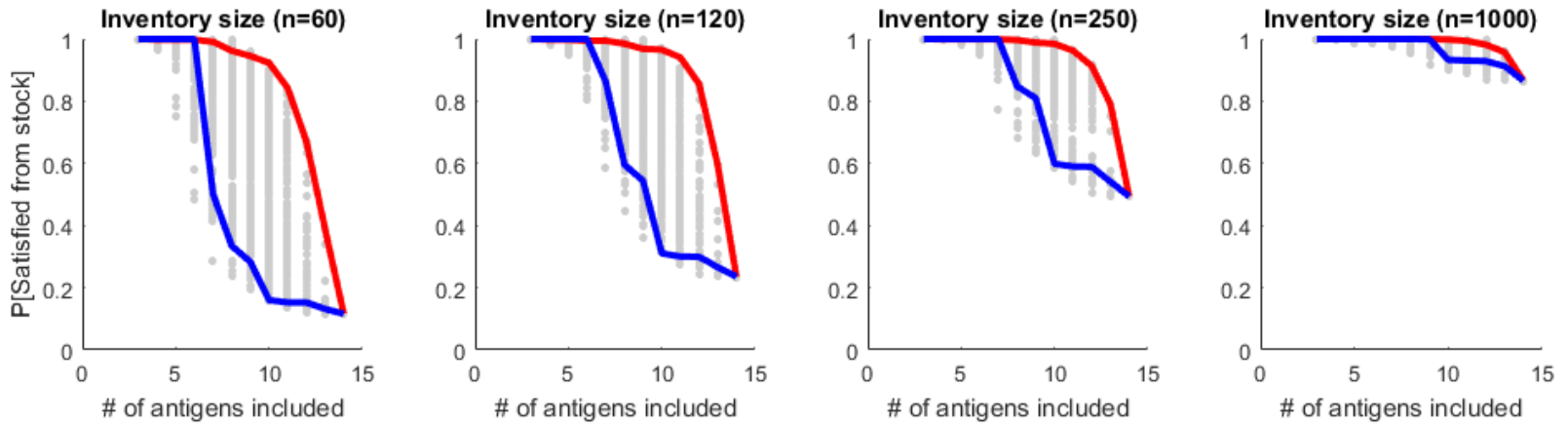
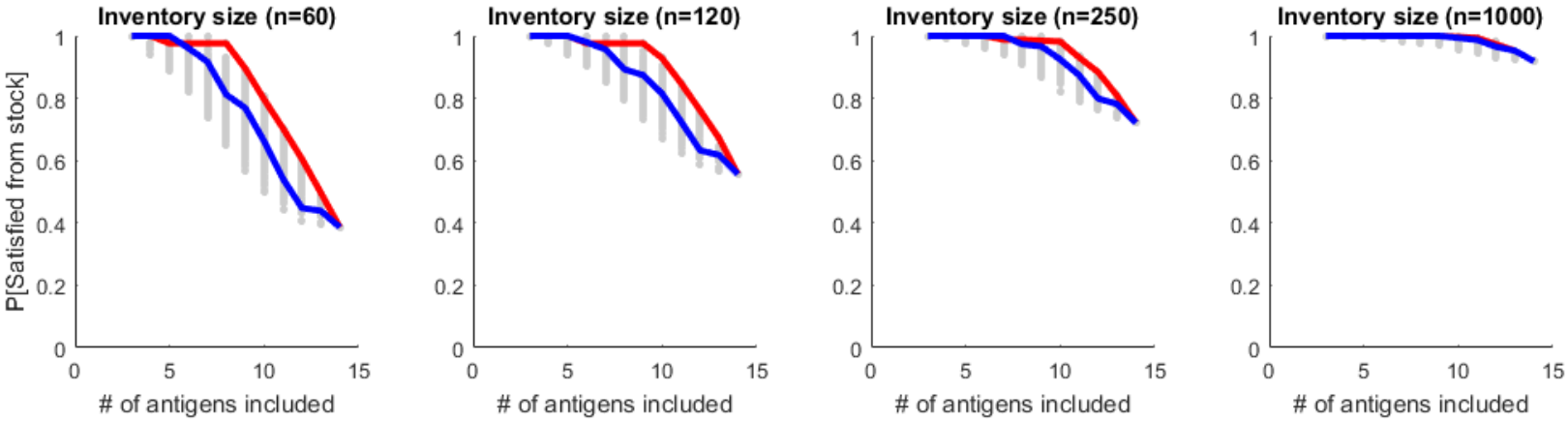
African patients <-> European donors

Requests fulfilled

Afr ↑ Eur



Eur ↑ Eur

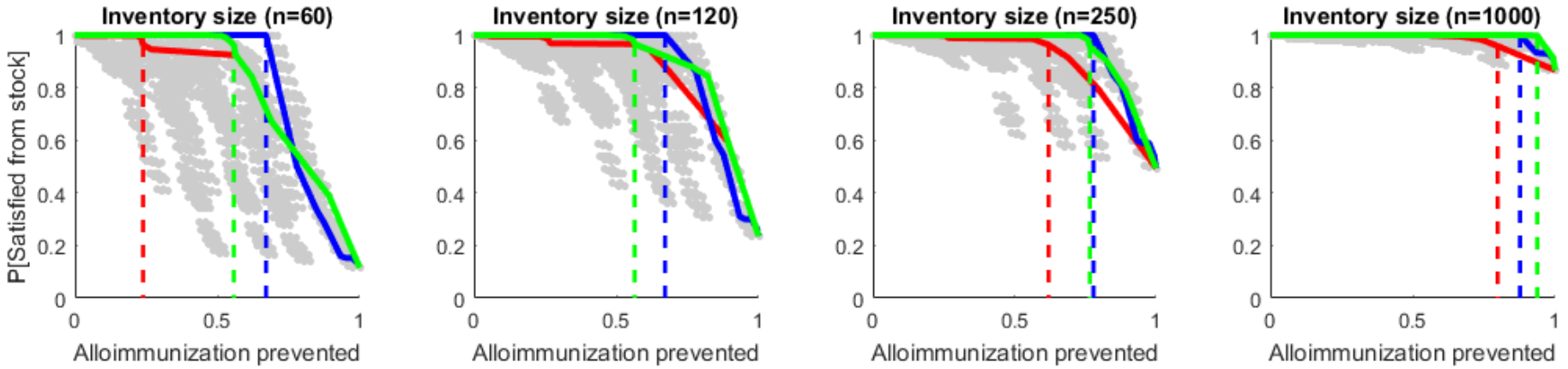


Other matching strategy for African patients

1. Maximize the percentage of requests that can be fulfilled directly from stock
2. Maximize the percentage of alloimmunisation prevented*
3. **Combination of strategy 1. and 2.**

$$\max_{\mathcal{I}} \sum_{i \in \mathcal{I}: F_i \geq p} c_i f_i \qquad \textbf{2.}$$

$$\text{s.t.} \sum_{i \in \mathcal{I}\ :\ F_i \geq p} f_i \geq 0.95 \qquad \textbf{1.}$$



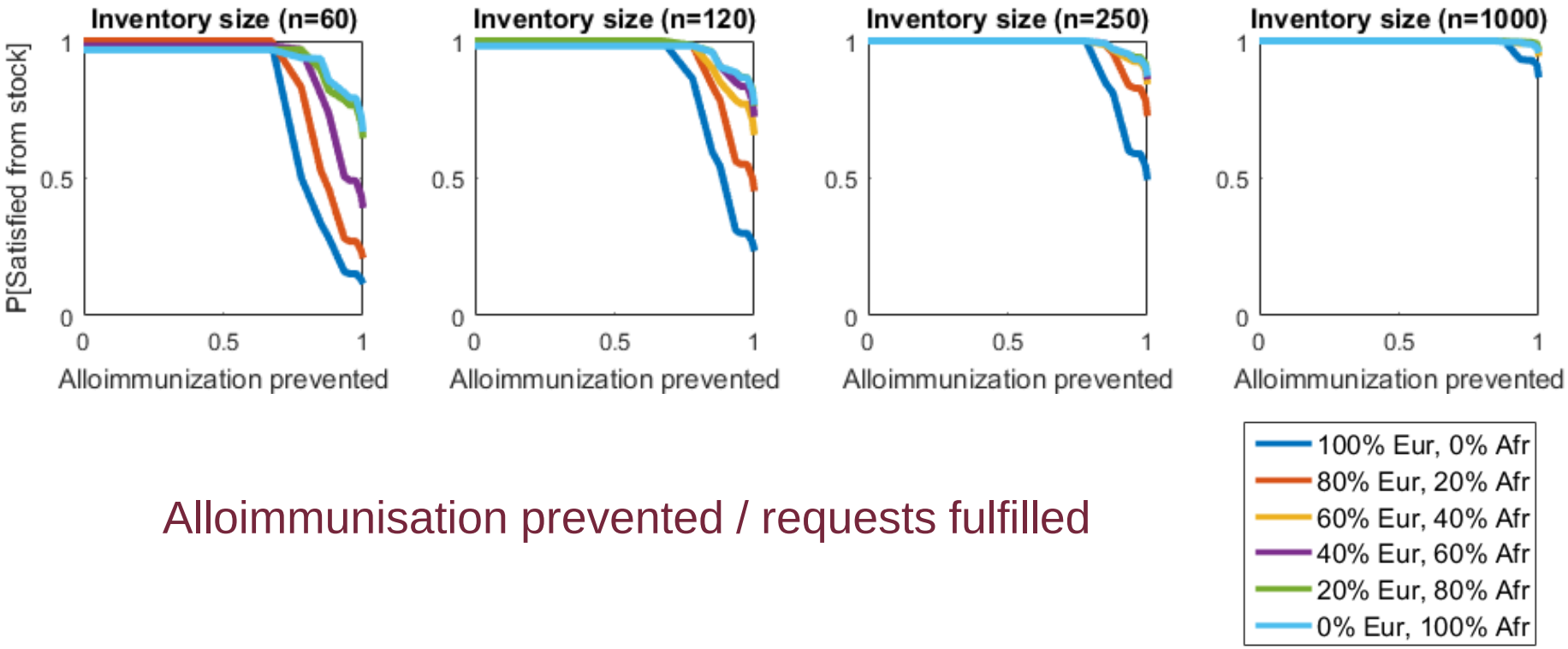
Alloimmunisation prevented / request fulfilled

ABO,D +E +K +e +c +Jk(a) +s +Fy(b)+Jk(b) +S +C +Fy(a)

** Schonewille, Thesis 2008, Leiden*

Effect of increasing the percentage of African donors is dependent on size of stock

- Patient population **100% African**



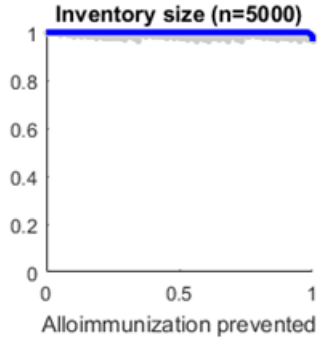
Alloimmunisation prevented / requests fulfilled

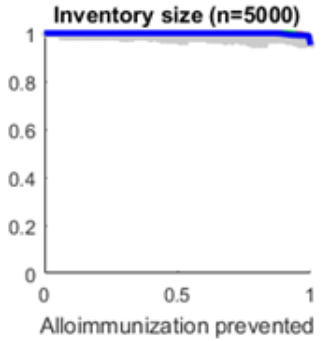
Conclusions (2)

- If all donors are fully genotyped, not only rare donors will be identified, also preventive matching becomes feasible

- >90% of alloimmunisation events can be prevented in a single patient
  - o 75% of European pts and 65% of African pts (stock = 60)
  - o 85% of European pts and 65% of African pts (stock = 120)
  - o 95% of European pts and 80% of African pts (stock = 250)
  - o 100% of European pts and 95% of African pts (stock = 1000)

E->E

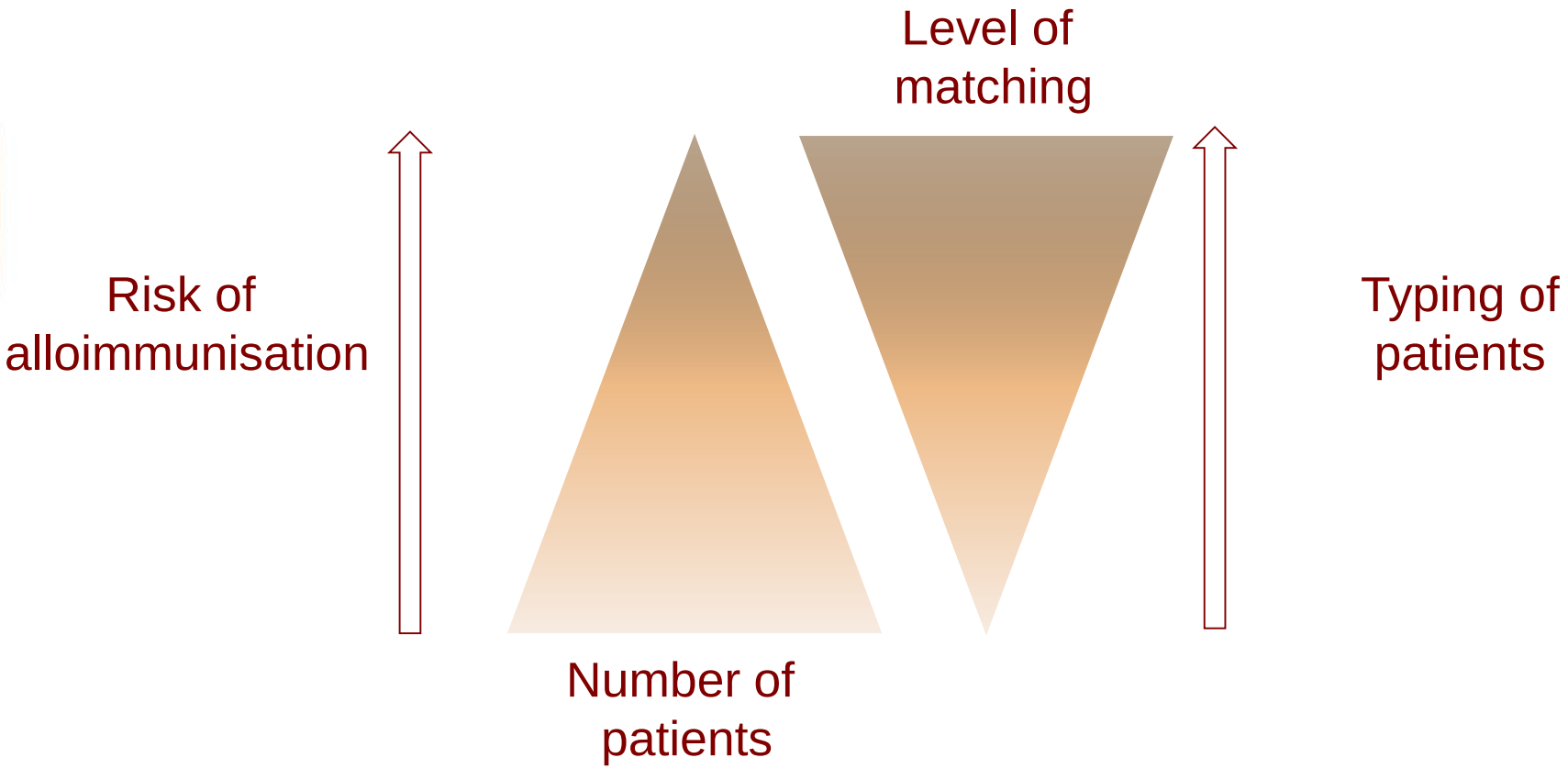




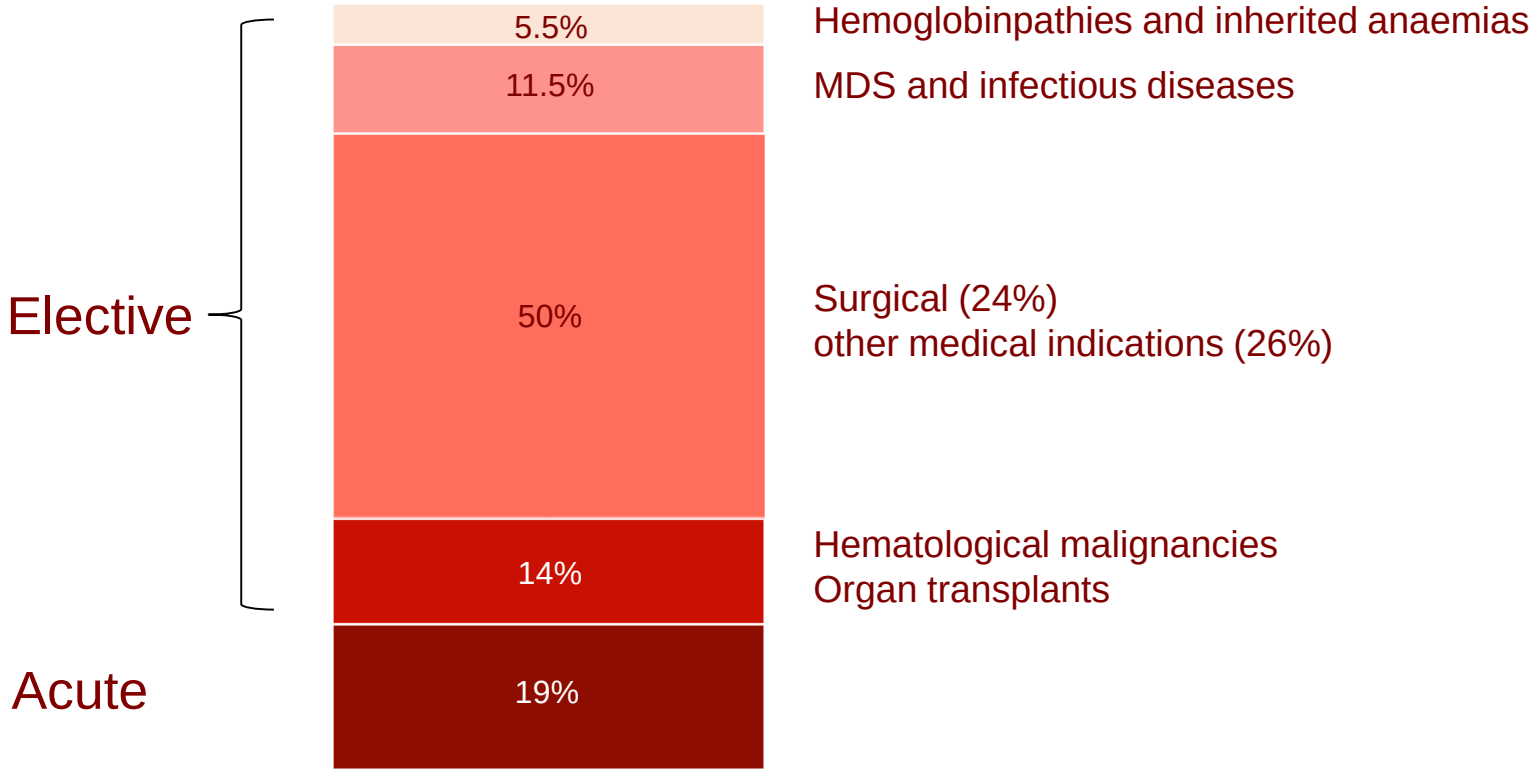
E->A Stock=5000

➢ We are currently constructing a dynamic model

Which patients should receive matched blood



Risk of alloimmunisation



Tinegate et al. Where do all the RBCs go? Transfusion 2016; 56:139-45

Evers et al. Hematologic malignancies associated with reduced red cell alloimmunization. Haematologica.2017;102:52-59
Evers et al. Red cell alloimmunisation in patients with different types of infections. Br J Haematol. 2016 ;175:956-966

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Bloedvoorziening

Final concusions

- Availability of SNV arrays optimized for comprehensive blood grouping and extended with more donor information will enable
  - to obtain fully typed donor cohorts
  - to identify rare donors
  - to perform ‘precision donation’

- This will make preventive matching possible at a larger scale
  - Logistics of blood supply might need to change:
    - Larger distribution centers from which blood for elective (planned) transfusions can be supplied
    - Algorithms to supply blood based on phenotypes

  - Patients should be typed more comprehensively
    - Serology for most immunogenic antigens
    - Extraction of data from WGS or WES data
    - (Recognition of high responders)

BloodMatch project team

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LRG
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Matching strategy Caucasian patient

- Patient population **100% Caucasian**, Donor population **100% Caucasian**
- 2 Matching strategies
  1. **Maximize the percentage of requests that can be satisfied directly from inventory**
  2. **Maximize the percentage of alloimmunization prevented**
  3. **Combination of strategy 1. and 2.**

# antigens	3	4	5	6	7	8	9	10	11	12	13	14
Strategy 1 ($n = 100$)	ABO, D	+e	+K	+E	+C	+s	+Fy(b)	+c	+Jk(a)	+Fy(a)	+Jk(b)	+S
Strategy 2	ABO,D	+E	+K	+Jk(a)	+c	+Fy(a)	+C	+S	+Jk(b)	+Fy(b)	+e	+s
Strategy 3 ($n = 100$)	ABO,D	+E	+K	+e	+C	+Jk(a)	+c	+s	+Fy(b)	+Fy(a)	+Jk(b)	+S

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Matching strategy African patient

- Patient population **100% African**, Donor population **100% Caucasian**
- 2 Matching strategies
  1. **Maximize the percentage of requests that can be satisfied directly from inventory**
  2. **Maximize the percentage of alloimmunization prevented**
  3. **Combination of strategy 1. and 2.**

# antigens	3	4	5	6	7	8	9	10	11	12	13	14
Strategy 1 ($n = 100$)	ABO, D	+e	+c	+Jk(a)	+s	+Fy(b)	+K	+E	+Jk(b)	+S	+C	+Fy(a)
Strategy 2	ABO,D	+E	+C	+K	+Fy(a)	+Jk(b)	+S	+Fy(b)	+e	+c	+Jk(a)	+s
Strategy 3 ($n = 100$)	ABO,D	+E	+K	+e	+c	+Jk(a)	+s	+Fy(b)	+Jk(b)	+S	+C	+Fy(a)