Blood transfusion safety: from mass-scale red cell genotyping to new antigens

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Disclosure

In relation to this presentation, I declare the following real or perceived conflicts of interest:

- Grant/Research Support:
  - Commonwealth Transfusion Foundation
  - Americas Blood Centers
- Speaker's honoraria:
  - Grifols SA
  - Ortho Clinical Diagnostics

A conflict of interest arises when speakers/authors have interests that are not fully apparent and that may influence their judgement in such a way that an independent observer might reasonably question whether the statements made are influenced by their own interests, and or that which, when revealed later, would make a reasonable reader/member of the audience feel misled or deceived. They may be personal, commercial, political, academic or financial. Financial interests may include employment, research funding and stock or share ownership, payment for lectures or travel, consultancies and company support of staff.

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Objectives

Upon completion of this presentation, participants will be able to:

Review how licensed serological reagents and genotyping lead to the discovery of variant antigens

Understand how blood group discrepancies can be an indication of an underlying acquired disease

Describe the difference between antigen matching and genotype (allele) matching

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Mass-scale red cell genotyping

Versiti's RCG program and implications
for the historical labeling
of
antigen-negative units

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Relative costs

Greg to Lorri circa Nov 2006:
“Lor can I buy a plasma TV?”
Lorri: “I guess so.”

Panasonic Viera $3500.00
Sound system $1500.00
Later that day Lorri:
“What were you thinking?”

Best Seller

$329.00

Qty | Ship this item | Qty | Pick up in club

Free shipping for *Plus*
Enter a ZIP Code

Pick it up today
Franklin, WI
Check more clubs

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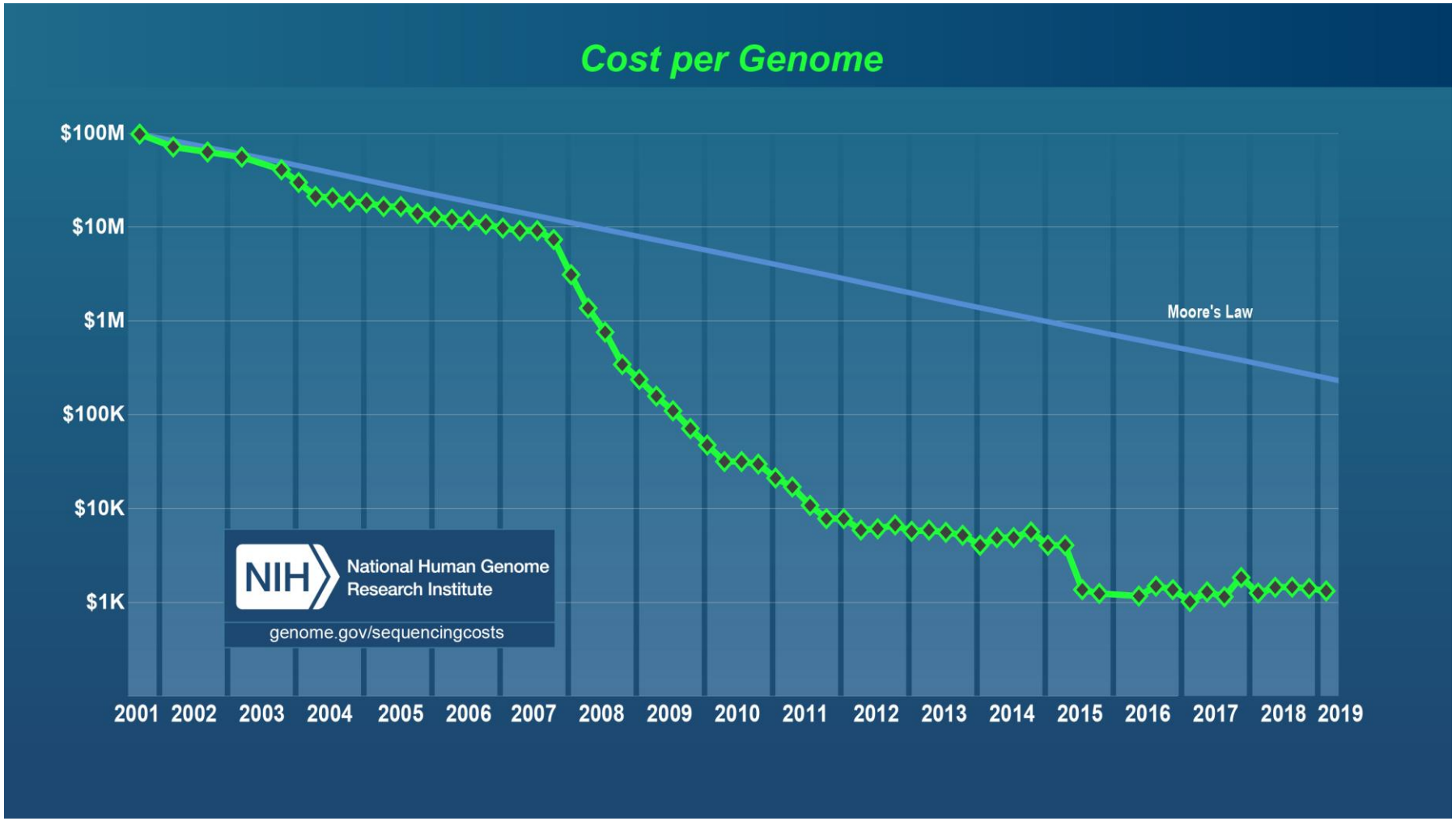
Human genome costs – SNP costs (pennies per SNP)



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AABB Standards and FDA guidance

AABB Standards for Blood Banks and Transfusion Services, 30th ed. 2016

5.8.4 Red Blood Cell Antigens other than ABO and RhD

- RBC units may be labeled as RBC antigen-negative, without testing the current donation, if two previous separate donations were tested by the collection facility and results of RBC typing were found to be concordant.

FDA guidance (Jan 2019) states the following options :

Directly on the bag label (follow ISBT 128)

- FDA-licensed only allowed, no need to indicate if the current unit was tested or was labeled from historical results

On a tie-tag affixed to the unit

- Unlicensed tests use this option and indicate whether the antigens are historical

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Mass-scale red cell genotyping

To be continued…

- Donor ID: Is this person the correct donor
- Upgrade to BECS to handle repeat serologic testing
- Alignment of serology (and molecular testing)

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Versiti:Wisconsin (Blood Center of Wisconsin)

Image used with permission

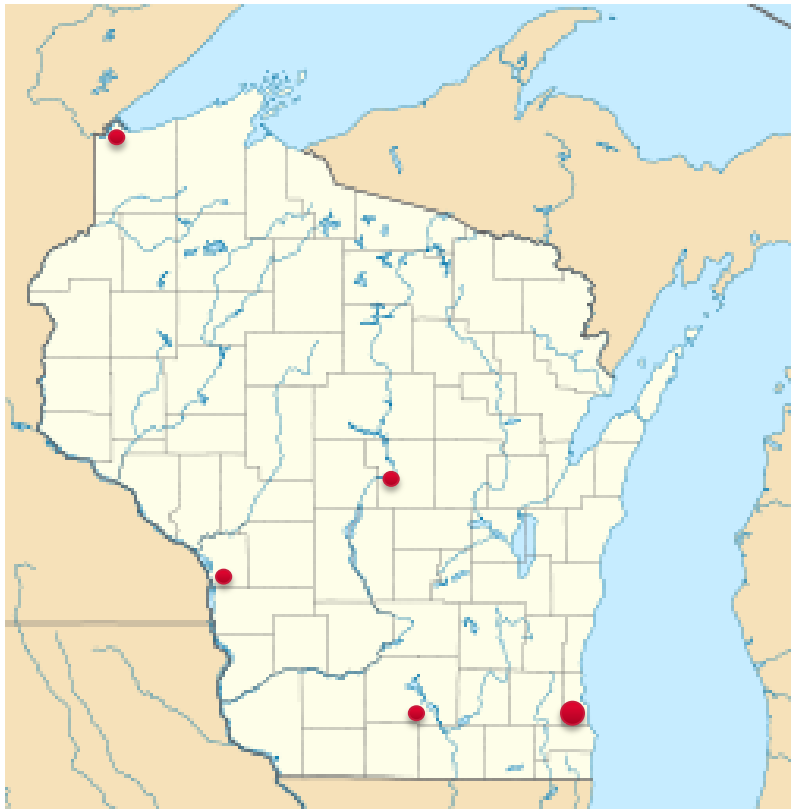
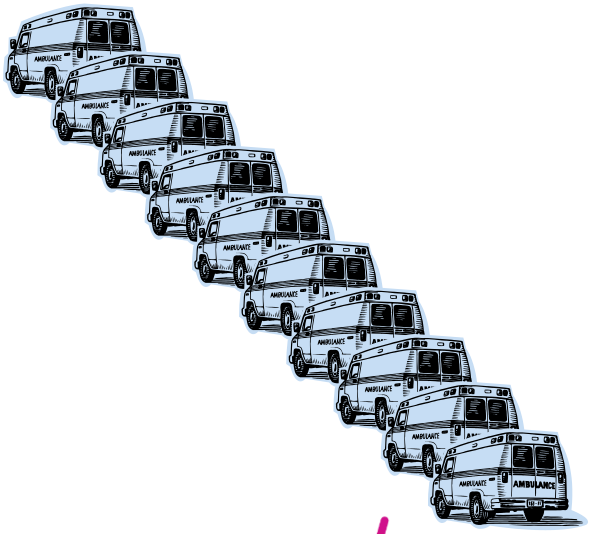


Image courtesy of en.wikipedia.com

Versiti:Wisconsin provides blood to some 64 hospitals serving a population of 3.7 million people



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What problem are we trying to solve?

- Genotyping has the potential to change how blood centers provide antigen-negative blood
  - To support alloimmunized patients:
    - multiple Abs, rare blood, antigen-pos with an Ab (variants)
    - These patients require genotype-matched blood (hrB/hrS)
  - To prevent alloimmunization:
    - SCD, WAIHA, other chronically transfused patients (MM)
- High-throughput mass-scale SNP analysis
  - SNPing shares a common chemistry
  - uses synthetic reagents
- Genotype is more accurate than a phenotype

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Cost of prevention

Kacker S, Ness PM, Savage WJ, *et al.* Economic evaluation of a hypothetical screening assay for alloimmunization risk among transfused patients with sickle cell disease. *Transfusion* 2014 Aug;54(8):2034-44.

- Cost of preventing alloimmunization $1.7b (USD)

Nickel RS, Hendrickson JE, Fasano RM, *et al.* Impact of red blood cell alloimmunization on sickle cell disease mortality: a case series. *Transfusion* 2015 Oct 28. doi: 10.1111/ trf.13379

- 5 SCD case reports of death due to alloimmunization

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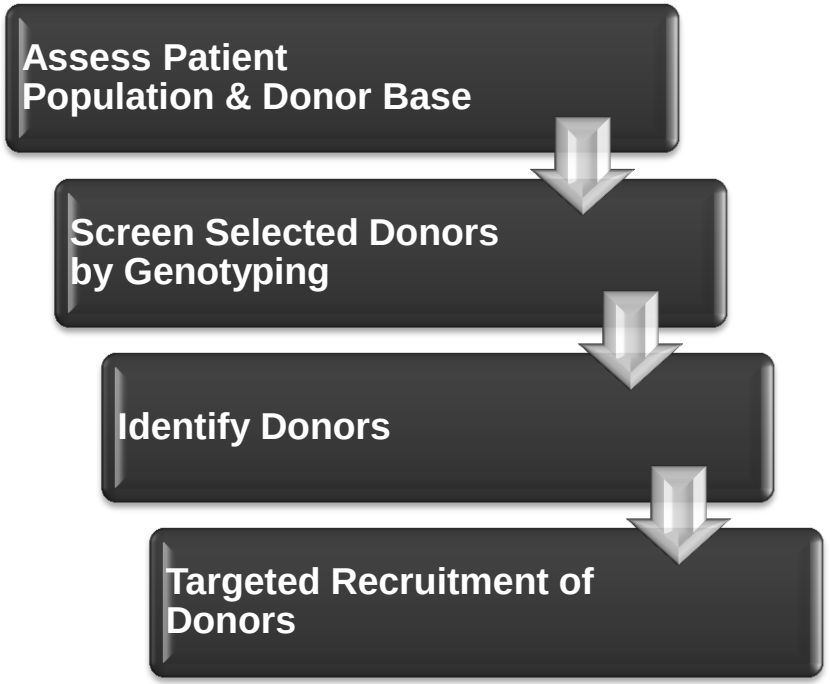
Red cell genotyping program

Red cell genotyping uses DNA to evaluate genes that predict particular red cell antigens

- **Donor Selection**
  - Optimize donor selection process
- **Donor Genotyping**
  - Obtain comprehensive results (low data loss)
  - Develop exception reports
  - Integrate interpretations (variants)
- **Data Management**
  - Maintain active database
  - Flexible targets (e.g. Vel)
- **Donor Recruitment**
  - Develop a registry maintenance strategy



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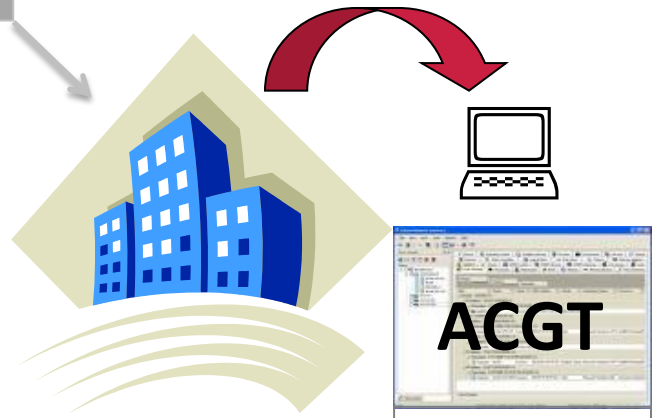
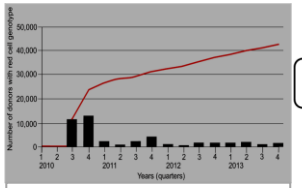
Clip Art



Hospital Transfusion Service

Antigen (Queries)



Donors (3:3:1:1)

Process

Import OpenArray files

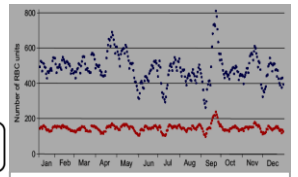
Apply call rules – genotypes to phenotypes

Parse Data

Confirm and Load Results to LIMS

Comparative Analysis of Results

Database



% RCG units

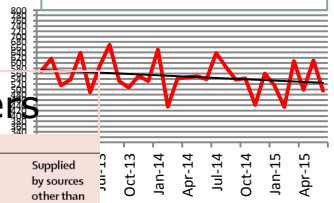
Mass-scale testing

Red Cell Genotyping

Outcomes

Evaluation

Utilization



Patient Encounters

Supplied in Wisconsin

	Patients	Patient encounters	Red cell units from inventory			Supplied by sources other than BloodCenter of Wisconsin
			Units identified by genotype only	Units identified by phenotype	Total units identified	
Number of negative antigens per unit requested						
1	612	1528	3473 (97·5%)	88 (2·5%)	3561	0
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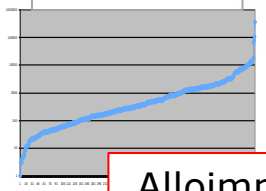
7	9	73	106 (68·4%)	49 (31·6%)	155	0
8	1	30	[illegible]	0	61	0
9	[illegible]	0	[illegible]	0	0	0
10	0	[illegible]	[illegible]	0	0	0
Rare‡	25	86	[illegible]	1 (0·7%)	135	21§
Total	1623	5672	[illegible]	9 (5·0%)	15 106	34

0
134 (99%)
14 357 (95%)

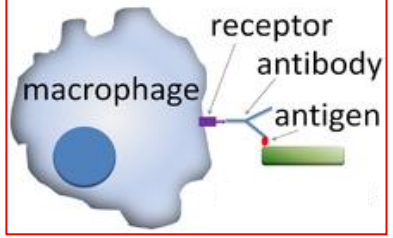
Recruitment

Requests

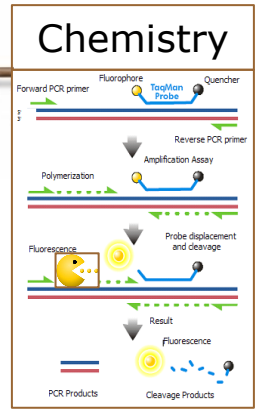


Targets

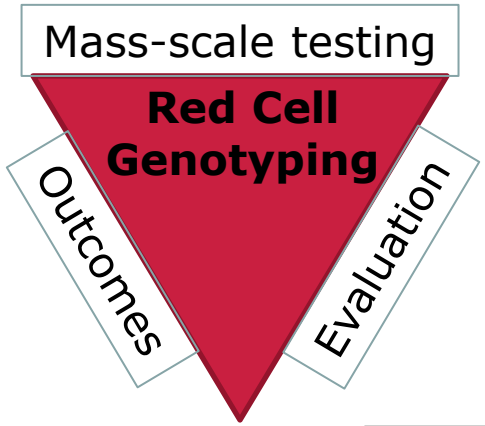
Alloimmunisation

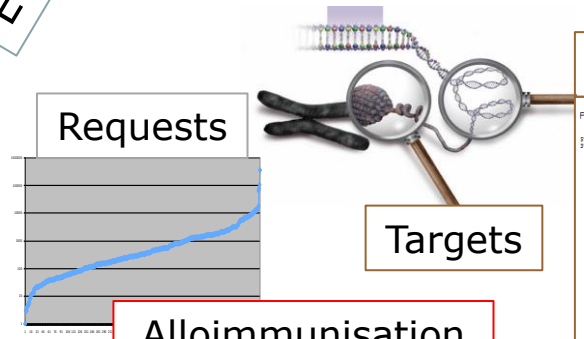


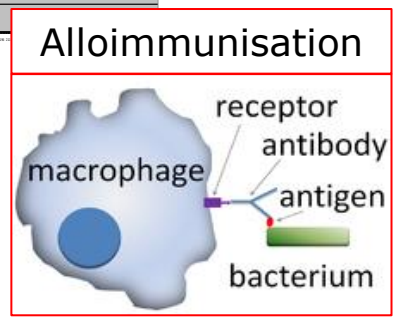
Chemistry

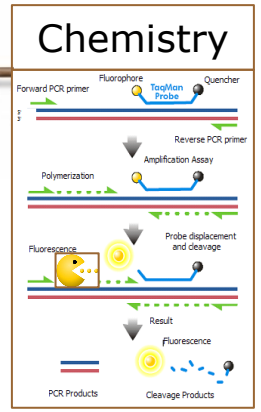


Images courtesy of en.wikipedia.com





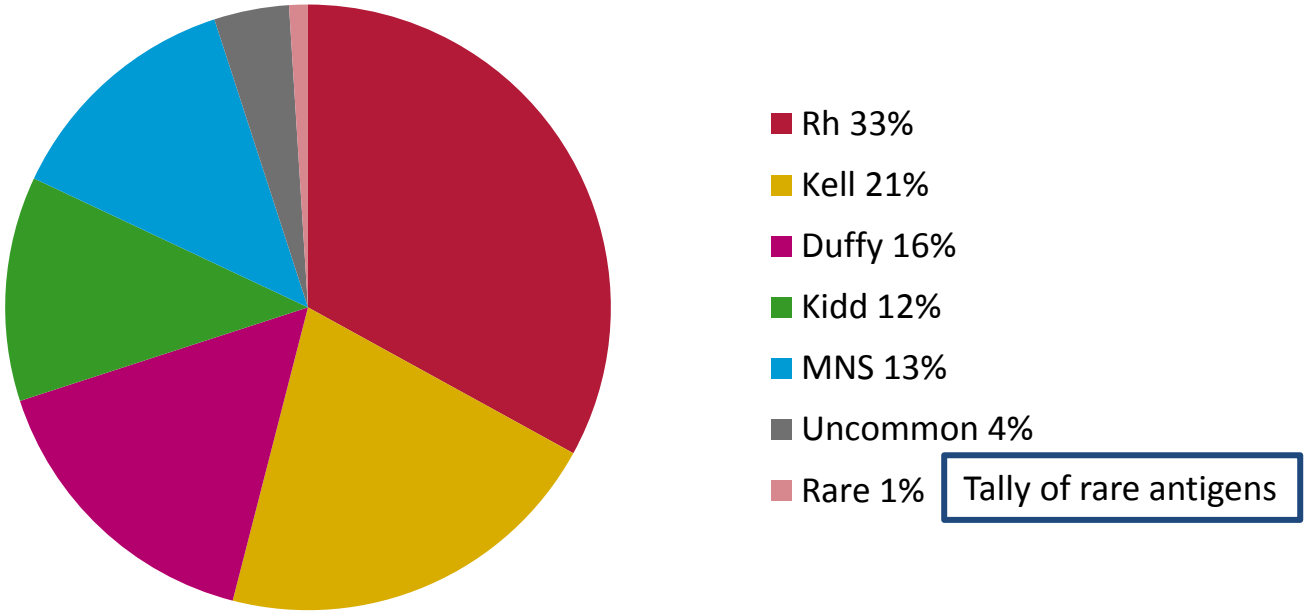




Images courtesy of wikipedia.com

Alloimmunization – which antigens?

1634 Antigen-negative Requests by Blood Group System



Multiple antibody combinations are common

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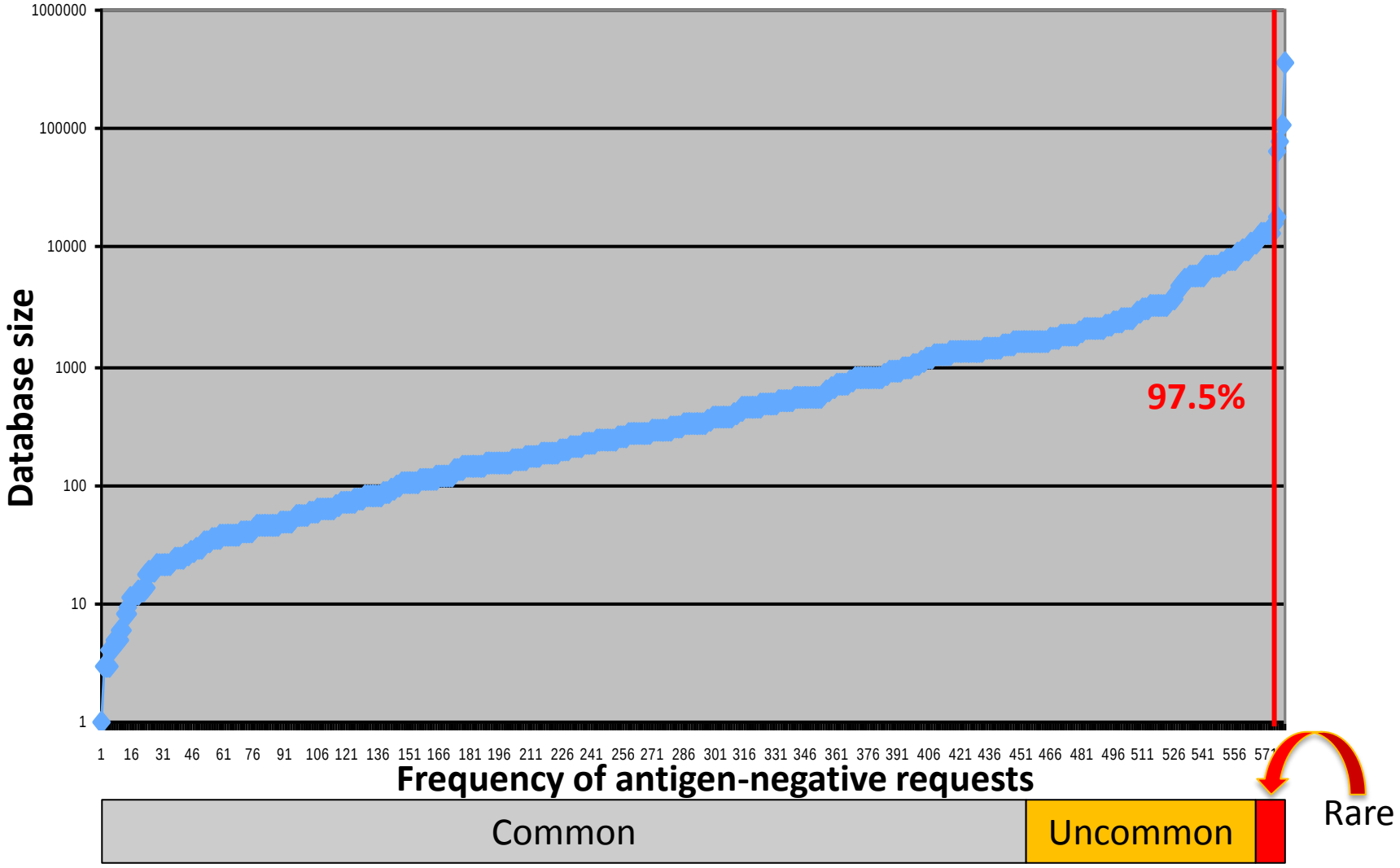
Goal of antigen-negative searches



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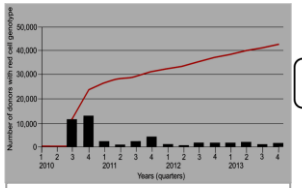


Donors
(3:3:1:1)

Process

Import OpenArray files

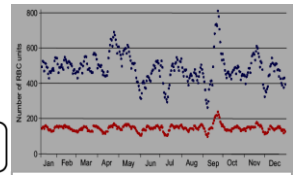
Apply call rules – genotypes to phenotypes

Parse Data

Confirm and Load Results to LIMS

Comparative Analysis of Results

Database



% RCG units

Mass-scale testing

Red Cell Genotyping

Outcomes

Evaluation

Phenotype:Genotype Relationship

System	Antigen	Gene	SNP rs#	NT Change	AA Change	VIC	FAM
Rh	E/e	*RHCE*	609320	C>G	P226A	G	C
Duffy	Fy^a/Fy^b	*FY*	12075	A>G	G42A	A	G
	Fy	*FY*	2814778	A>G	promoter	G	A
	Fy^x	*FY*	34599082	G>A	R89C	G	A
Kidd	Jk^a/Jk^b	*JK*	1058396	A>G	D280N	A	G
Lutheran	Lu^a/Lu^b	*LU*	28399653	A>G	R77H	A	G
Kell	Js^a/Js^b	*KEL*	8176038	C>T	L597P	T	C
	K/k	*KEL*	8176058	T>C	M193T	C	T
Dombrock	Do^a/Do^b	*DO*	11276	A>G	N265D	G	A
	Jo^a	*DO*	28362798	C>T	T117I	C	T

Ag	Target	Genotype	Phenotype	Comment
RhC	IVS2+109 RH2+	C+	$(C)ce^S$	
RhE	676C	RH3+	E+	E type III
Rhc	307C	RH4+	c+	
Rhe	676G	RH5+	e+	ceMO

Molecular basis of blood group expression. *Transf Apher Sci* 2011;44:53-63

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BECS: Phenotype (common) Genotype (ISBT)

Antigen	ISBT terminology		Antigen	ISBT terminology	
	Number	Symbol		Number	Symbol
M	002.001	MNS1	Js^a	006.006	KEL6
N	002.002	MNS2	Js^b	006.007	KEL7
S	002.003	MNS3	Fy^a	008.001	FY1
s	002.004	MNS4	Fy^b	008.002	FY2
U	002.005	MNS5	Jk^a	009.001	JK1
C	004.002	RH2	Jk^b	009.002	JK2
E	004.003	RH3	Jk3	009.003	JK3
c	004.004	RH4	Di^a	010.001	DI1
e	004.005	RH5	Di^b	010.002	DI2
V	004.010	RH10	Yt^a	011.001	YT1
hrS	004.019	RH19	Yt^b	011.002	YT2
VS	004.020	RH20	Sc1	013.001	SC1
hrB	004.031	RH31	Sc2	013.002	SC2
Crawford	004.043	RH43	Do^a	014.001	DO1
Lu^a	005.001	LU1	Do^b	014.002	DO2
Lu^b	005.002	LU2	Hy	014.004	DO4
Lu8	005.008	LU8	Jo	014.005	DO5
Lu14	005.014	LU14	Co^a	015.001	CO1
K	006.001	KEL1	Co^b	015.002	CO2
k	006.002	KEL2	Cr^a	021.001	CROM1
Kp^a	006.003	KEL3			
Kp^b	006.004	KEL4			

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RBC Genotyping Donor Selection

Definition of a repeat donor - used a 3:3:1:1 rule

- On the day of selection, donor has donated 3 times in the past 3 years, of which 1 donation was within the previous calendar (1) year

Definition of a valued donor:

- Screen low repeat and first time donors
  - Genotype all R_0, R_2, and 10% R_1 donors
  - Goal is to meet the need of the majority of local antigen-negative requests and provide units to rare unit programs (frozen blood, national program)

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Mass-scale data handling

Import OpenArray files
- Data from instrument dropped into server folder
- Raw data validated (expected fields)

Apply call rules – genotypes to predicted phenotypes
- Apply logic rules to individual SNPs
- Apply complex rules involving multiple SNPs

Parse Data
- Parse the data from genotype to predicted phenotype
- Operator Inspects translated data

Confirm and Load Results to LIMS
- Data sorted on the basis of sample accession number

Comparative Analysis of Results
- Apply queries to display predicted phenotypes
- Apply exception reports; extract and display results

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Blood donor phenotypes and genotypes

Phenotyping
30 years

638,786 donors
1985 – 2013
(91% from 1991)

	Tested by phenotype		Tested by genotype†	
1	20 147	20 147	0	0
3	7016	21 048	0	0
4	7850	31 400	0	0
5	5222	26 110	0	0
6	2990	17 940	0	0
7	2221	15 547	0	0
8	3830	30 640	0	0
9	2367	21 302	0	0
10	1535	15 350	0	0
11	899	9889	0	0
12	805	9660	0	0
13	1432	18 616	0	0
14	1656	23 184	0	0
15	914	13 710	0	0
16	691	11 056	0	0
17–25	678	12 479	0	0
26–28	6	160	5	137
29–33	0	0	364	11 688

Genotyping
4 years

202,275 donors
2010 – 2013
(16% yr-over-yr)

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Advantage of genotyping over phenotyping

638,786 donors
1985 – 2013
(91% from 1991)

	Tested by phenotype		Tested by genotype†	
26–28	6	160	5	137
29–33	0	0	364	11 688
34	0	0	878	29 852
35	0	0	539	18 865
36	0	0	3916	140 976
37	0	0	1260	46 620
38	0	0	21 144	803 472
39	0	0	995	38 805
40	0	0	1033	41 320
41	[illegible]	[illegible]	7853	[illegible]
42	[illegible]	[illegible]	50[illegible]	[illegible]
Total	72[illegible]	[illegible]	430[illegible]	[illegible]
Donors not te[illegible]	523 3[illegible]	[illegible]	595 72[illegible]	[illegible]

0
322264

213318
1667026

202,275 donors
2010 – 2013
(15% yr-over-yr)

Historical	Donors	Phenotypes	Antigens	Years
Phenotypes	72,272	322264	1 - 28	30
Genotypes	43,066	1667026	28 - 42	4

5×

1/7th

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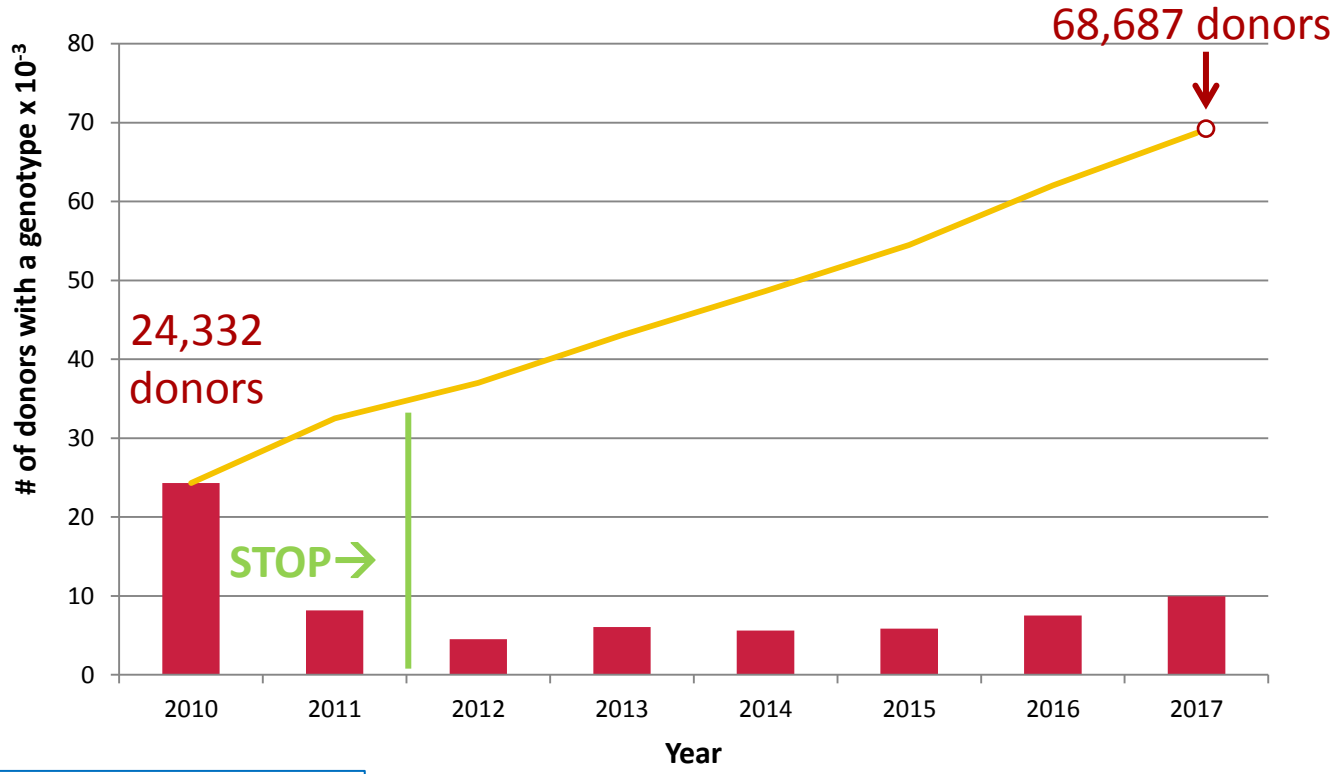
RCG Testing

Donor Red Cell Genotyping (2010 - 2017)



2.1 units/donor/yr

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Geno/phenotype units supplied in Wisconsin

	Supplied in Wisconsin					
	Patients	Patient encounters	Red cell units from inventory			Supplied by sources other than BloodCenter of Wisconsin
			Units identified by genotype only	Units identified by phenotype	Total units identified	
Number of negative antigens per unit requested						
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7	9	73	106 (68·4%)	49 (31·6%)	155	0
8	1	30	61 (100%)	0	61	0
9	[illegible]	0	[illegible]	0	0	0
10	0	[illegible]	[illegible]	0	0	0
Rare‡	25	86	[illegible]	1 (0·7%)	135	21§
Total	1623	5672	[illegible]	749 (5·0%)	15 106	34

5672 patient 'encounters'

0

134 (99%)

14 357 (95%)

34 units imported for 11 of 5672 patient encounters

Common antigens:
5 C-e-Jk(b-)
6 C-e-S-K-Jk(b-)
1 C-e-K-Fy(b-)Jk(b-)
1 E-K-Fy(a-b-)Co(b-)

Rare antigens:
10 E-Di(b-)
8 U- (E-K-)
1 E-S-K-Jk(b-)Yt(a-)
1 'N'
1 Bombay

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Geno/phenotype units supplied elsewhere

Supplied to elsewhere in the USA

Patients	Patient encounters	Red cell units from inventory		
		Units identified by genotype only	Units identified by phenotype	Total units identified
6	8	18 (75·0%)	6 (25·0%)	24
15	17	71 (100%)	0	71
26	27	68 (100%)	0	68
50	60	146 (98·6%)	2 (1·4%)	148
109	169	345 (98·6%)	5 (1·4%)	350
60	84	129 (83·8%)	25 (16·2%)	154
21	27	36 (72·0%)	14 (28·0%)	50
14	30	48 (82·8%)	10 (17·2%)	58
7	10	[illegible]	12 (75·0%)	16
2	[illegible]	[illegible]	5 (100%)	5
341	626	[illegible]	119 (9·9%)	1199
651	1062	[illegible]	198 (9·2%)	2143

1062 patient 'encounters'

0

1080 (90%)

1945 (91%)

Rare units

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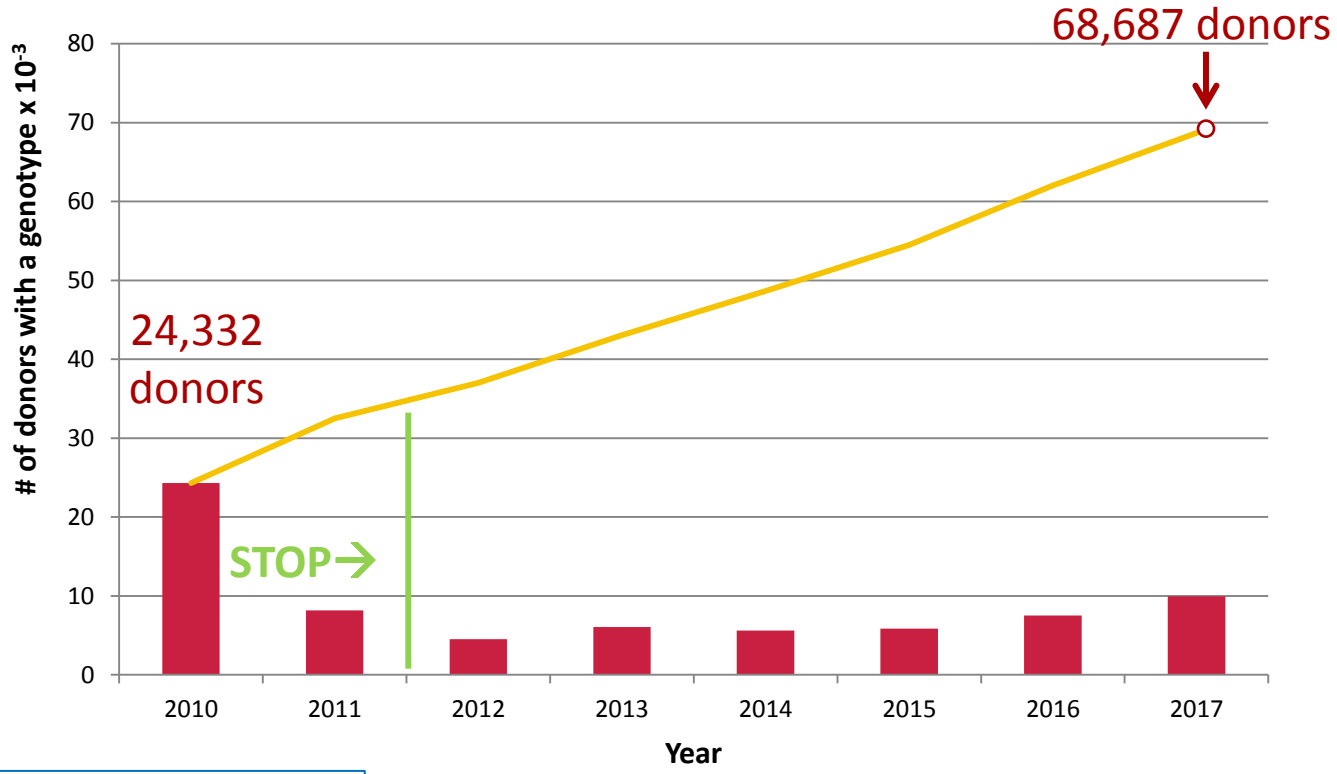
RCG Testing

Donor Red Cell Genotyping (2010 - 2017)



2.1 units/donor/yr

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Replacement donors - 3:3:1:1 rule

UNIT ID#	GENDER	ABO	UNITS
W036315414014-E	M	OPOS	14
W036315268275-O	M	OPOS	11
W036315060756-O	F	OPOS	10
W036315307323-*	M	OPOS	10
W036315033361-*	M	OPOS	9
W036315033358-Q	M	OPOS	8
W036315060733-1	M	OPOS	7
W036315115870-*	F	OPOS	7
…	…	…	…
W036315268272-U	M	OPOS	3
W036315417539-J	F	OPOS	3
W036315416121-D	M	ONEG	14
W036315033375-O	F	ONEG	14
W036315307361-O	M	ONEG	12
W036315060729-U	M	ONEG	11
W036315192085-Q	M	ONEG	10
W036315033400-H	F	ONEG	9
W036315115889-E	M	ONEG	9
W036315307316-Y	M	ONEG	9
W036315033401-F	F	ONEG	8

↑ **2.4 units/donor/yr**

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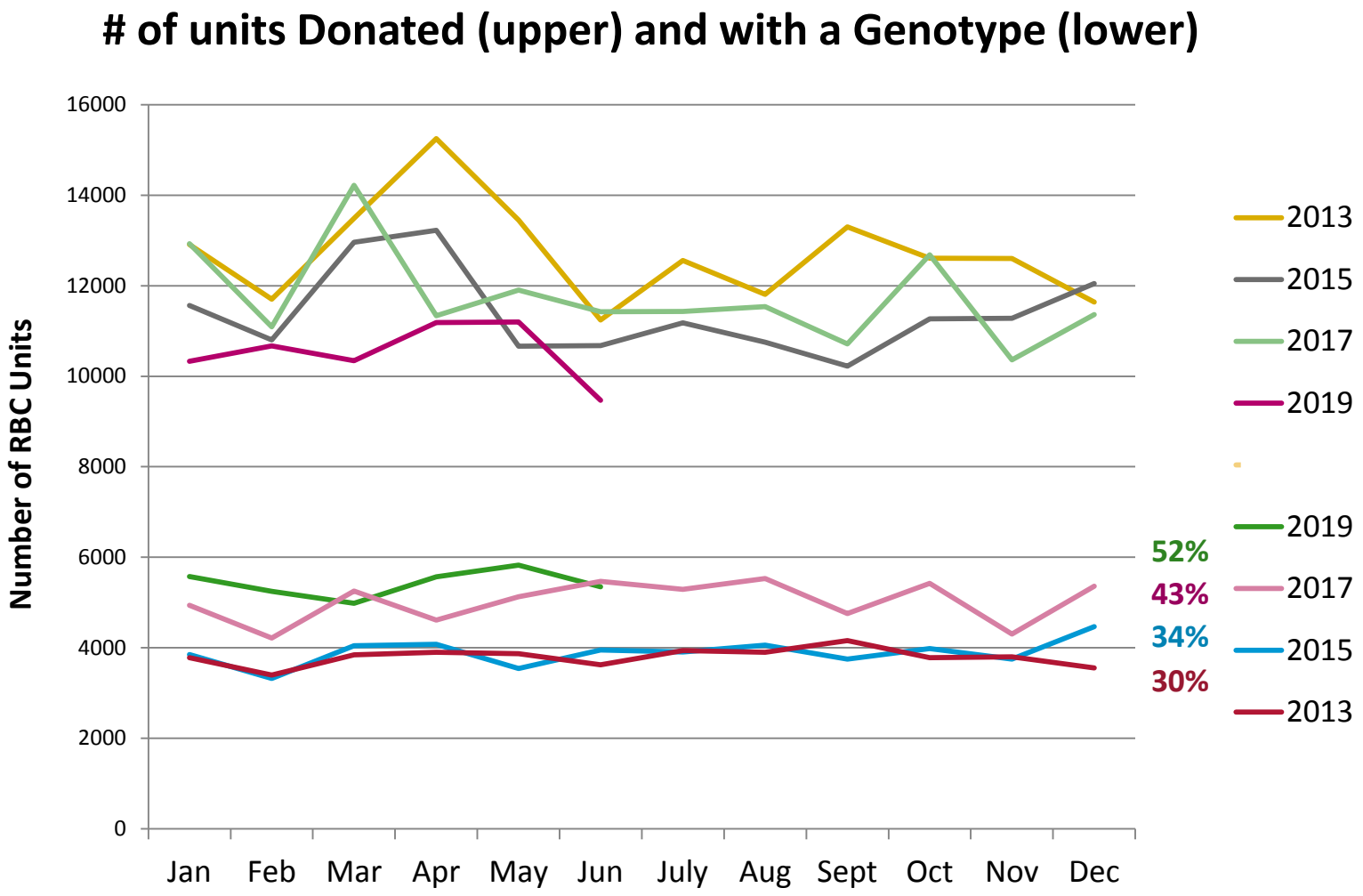
Daily impact of genotyping



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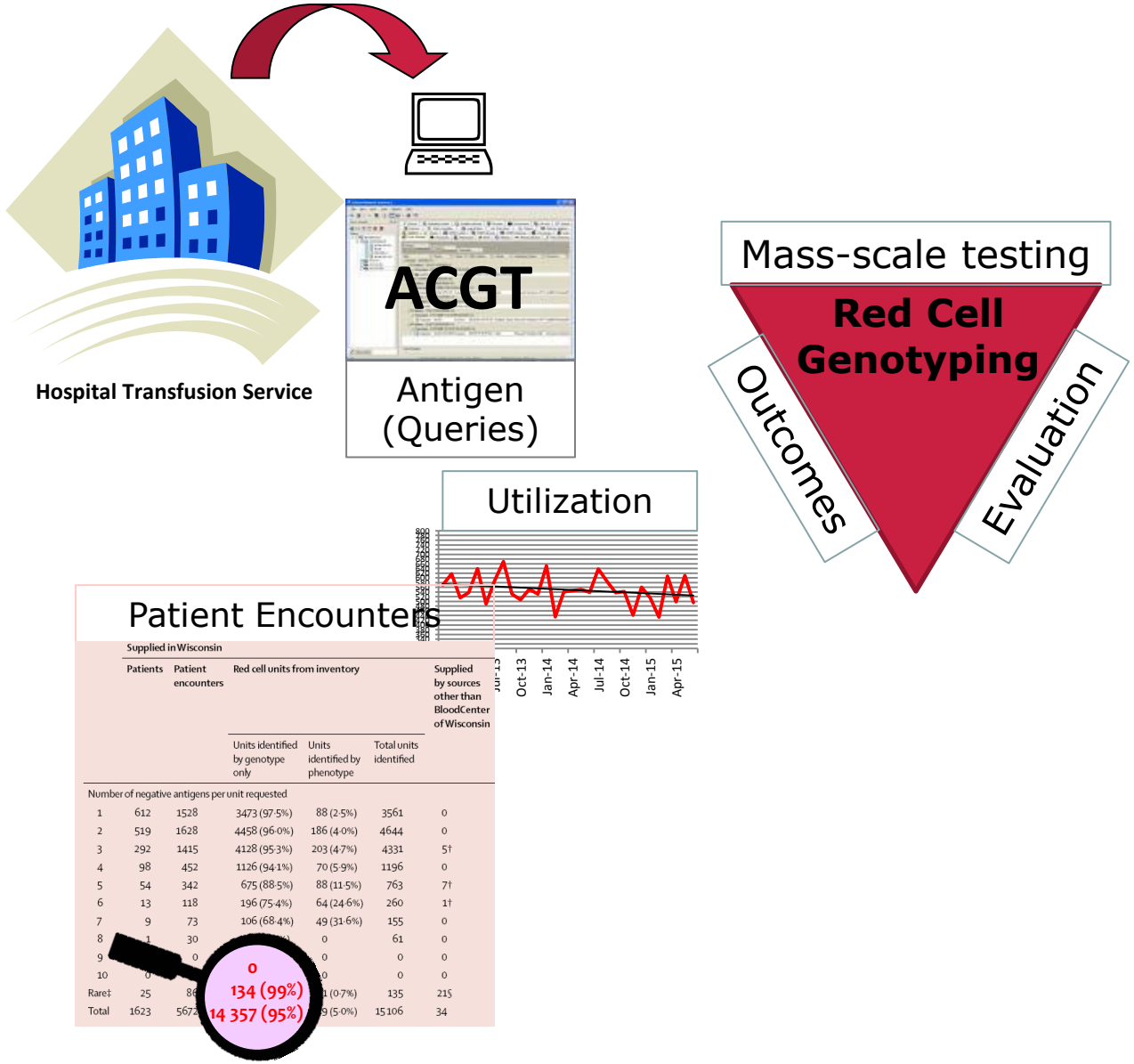
Hospital Transfusion Service
ACGT
Antigen (Queries)
Mass-scale testing
Red Cell Genotyping
Outcomes
Evaluation
Utilization
Oct-13
Jan-14
Apr-14
Jul-14
Oct-14
Jan-15
Apr-15
Patient Encounters
Supplied in Wisconsin
Patients
Patient encounters
Red cell units from inventory
Units identified by genotype only
Units identified by phenotype
Total units identified
Supplied by sources other than BloodCenter of Wisconsin
Number of negative antigens per unit requested
1 612 1528 3473 (97·5%) 88 (2·5%) 3561 0
2 519 1628 4458 (96·0%) 186 (4·0%) 4644 0
3 292 1415 4128 (95·3%) 203 (4·7%) 4331 5†
4 98 452 1126 (94·1%) 70 (5·9%) 1196 0
5 54 342 675 (88·5%) 88 (11·5%) 763 7†
6 13 118 196 (75·4%) 64 (24·6%) 260 1†
7 9 73 106 (68·4%) 49 (31·6%) 155 0
8 1 30 0 61 0
9 0 0 0 0
10 0 0 0 0
Rare‡ 25 86 1 (0·7%) 135 21§
Total 1623 5672 9 (5·0%) 15 106 34
0
134 (99%)
14 357 (95%)

Antigen Query – use of data

Portal transmits antigen information with a red cell unit by use of the ISBT 128 number

Antigens available to query: **C, E, c, e, M, N, S, s, K, Fya, Fyb, Jka, Jkb** (antigen-negative types most likely encountered based on inventory size)

In the initial rollout, 7 hospitals found 71 units in 52 queries

14 hospital blood banks use antigen query, with most centers 30 to 200+ miles away

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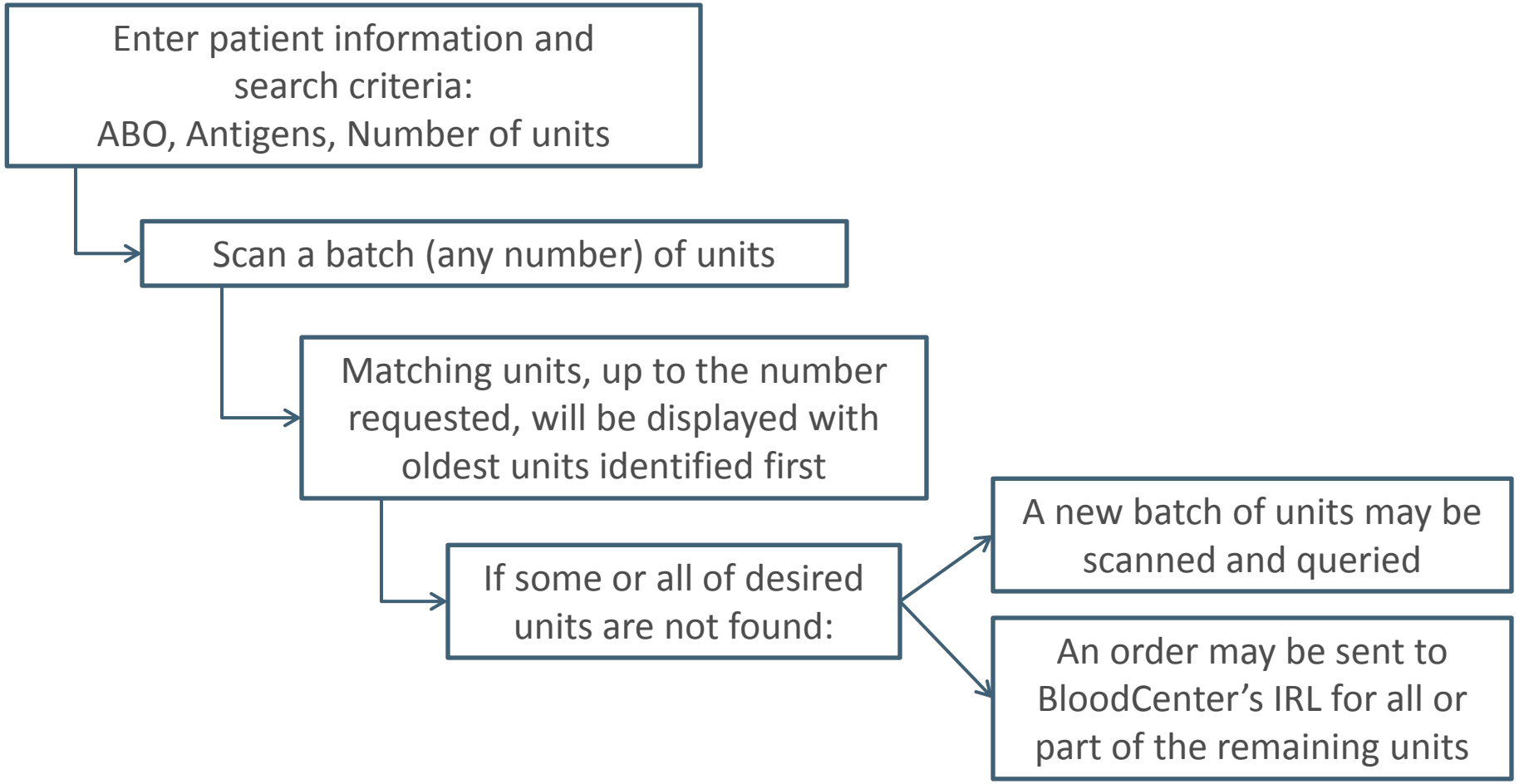
Antigen Query - workflow

The process closely mirrors Hospital's current practice to phenotype units



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Antigen Query: blood order portal

Unit Selection

127.0.0.1:7101/agq/chgHosp.jsf

Current: ST LUKES MEDICAL CENTER SLK (Change)

- Patient Query
- General Query
- Hospital Orders
- Activity (IRL View)
- Orders (IRL View)

Unit Selection - ST LUKES MEDICAL CENTER SLK

Patient Name

Contact IRL

Last * Smith First * John Mi A

DOB * 1/1/1980 Gender * M F Patient's ABO/Rh * O Pos

Admission No * PAT000123 Reason * Antigen Matching

Notes

* Please select at least one

☑ O pos ☑ A pos ☐ B pos ☐ AB pos ☐ O neg ☐ A neg ☐ B neg ☐ AB neg CMV IRR

* Please select at least one antigen negative checkbox

Rh	Kell	MNS	Kidd	Duffy
☐C	☑K	☐M	☑Jka	☐Fya
☐ c̄		☐N	☐Jkb	☐Fyb
☐E		☐S		
☐ ē		☐ s̄		
☐Cw				

* Please enter number of units to find

Units To Find 2

Scan / Enter Units Clear - Start Over Place Order

Patient-specific request

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Antigen Query Video Clip

Antigen(s)		f	Queries (n)*	Antigen Query with Phenotype†	Total Antigen Query‡	Proportional Difference	Total Phenotype Screen‡	Batch Time†	P(n,r,p) > 0, 0.95	Batch size (n)
One antigen	K-	0.910	248	30	7440	100	7440	30	3	3
	E-	0.710	628	30	18840	100	18840	30	3	3
	S-	0.450	91	30	2730	133	3640	40	5	6
	Fya-	0.340	304	30	9120	167	15200	50	7	9
	Jka-	**0.230**	**477**	**30**	**14310**	**200**	**28620**	**60**	**12**	**12**
	M-	0.220	36	30	1080	200	2160	60	12	12
	c-	**0.200**	**163**	**30**	**4890**	**250**	**12225**	**75**	**14**	**18**
	Fyb-	0.170	29	30	870	250	2175	75	16	18
	s-	0.110	3	30	90	350	315	105	26	36
Two antigens	E- K-	0.646	120	30	3600	100	3600	30	3	3
	Fya- K-	0.309	26	30	780	167	1300	50	8	9
	C- K-	0.291	48	30	1440	167	2400	50	9	9
	Jkb- K-	0.237	22	30	660	200	1320	60	11	12
	E- Jka-	**0.163**	**81**	**30**	**2430**	**250**	**6075**	**75**	**17**	**18**
	C- Fya-	0.109	10	30	300	350	1050	105	26	36
	C- Jka-	**0.074**	**18**	**30**	**540**	**400**	**2160**	**120**	**39**	**48**
	c- Jkb-	0.052	1	30	30	450	135	135	56	60
Three antigens	E- K- S-	0.291	26	30	780	167	1300	50	9	9
	C- E- K-	0.182	177	30	5310	250	13275	75	15	18
	C- K- S-	0.131	9	30	270	250	675	75	22	18

IRL inventory management – PK screening

- BCW reduced the number of IRL units held
  - 10 R_1R_1 10 R_2R_2 10 R_0R_0/R_0r 10 rr

    with multiple-antigen negative attributes, e.g. S- Fy(a-), Jk(a-)

- Improve management of local blood utilization
  - Limit the number of Rh-negative units going to Rh-positive transfusion recipients (red cell pheresis SCD program) who don't need them
  - D+C-E-K- replaces D-C-E-K-

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PK7300 Screening Daily Donations

Solution: Strategy to build Rh/Kell typed units

Data: # of daily donations for 2017 42 day outdate

Assumptions: 2017 daily red cell donations = 95% 2018

Includes AA , HI, AS donors

Includes Rh–negative donors

Assumes 40% of donors are 1st time/low

2.5% R_0 2% R_2 2% R_1(10% of total)

Minor ethnicities and Rh-negative donors are included!

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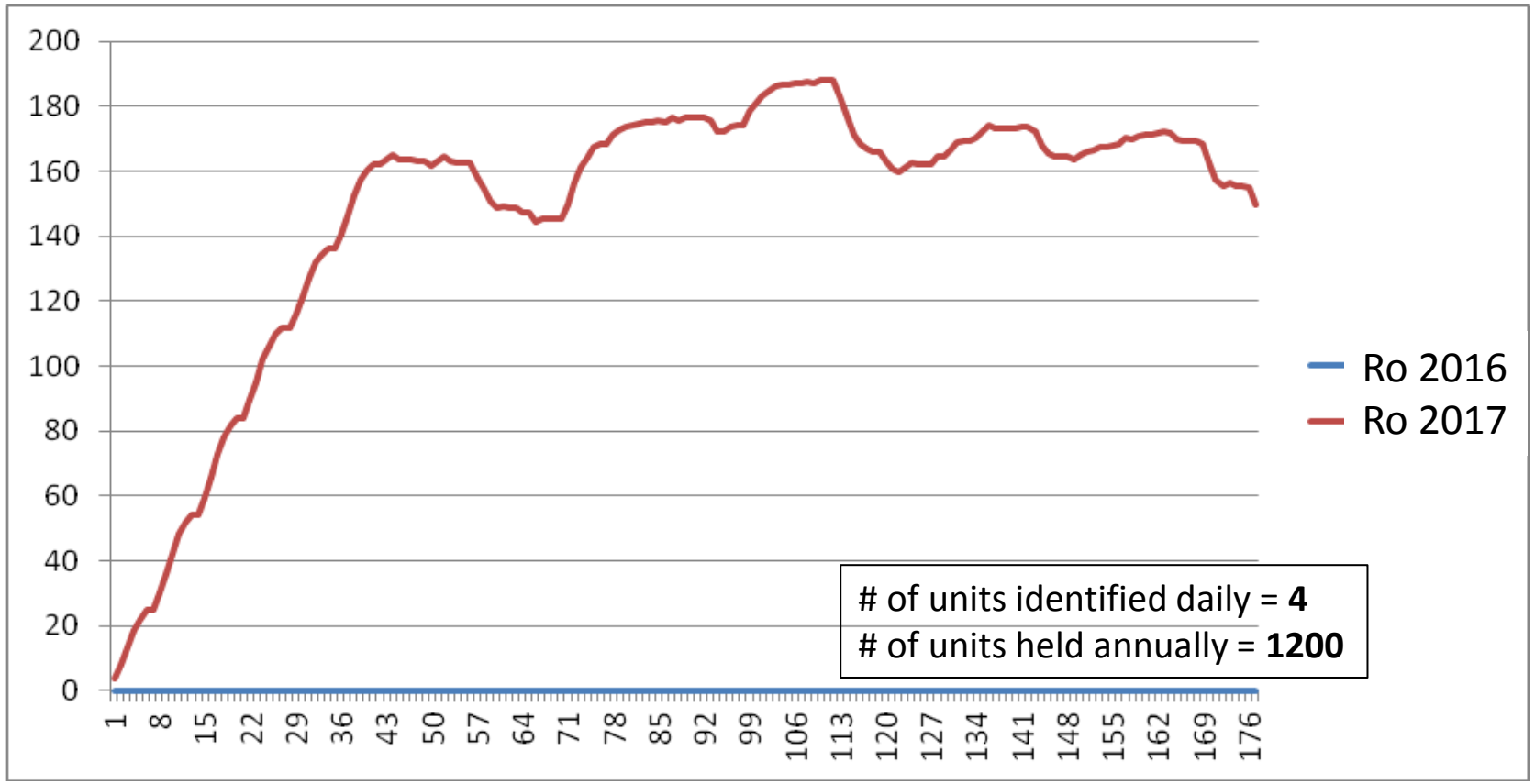
Figure 1. The estimated 42 day running total of serologically screened Ro, R2, R1 units diverted to a special inventory to support the need. N = 1200 units annually



Daily monitoring: data entered into a dynamic worksheet

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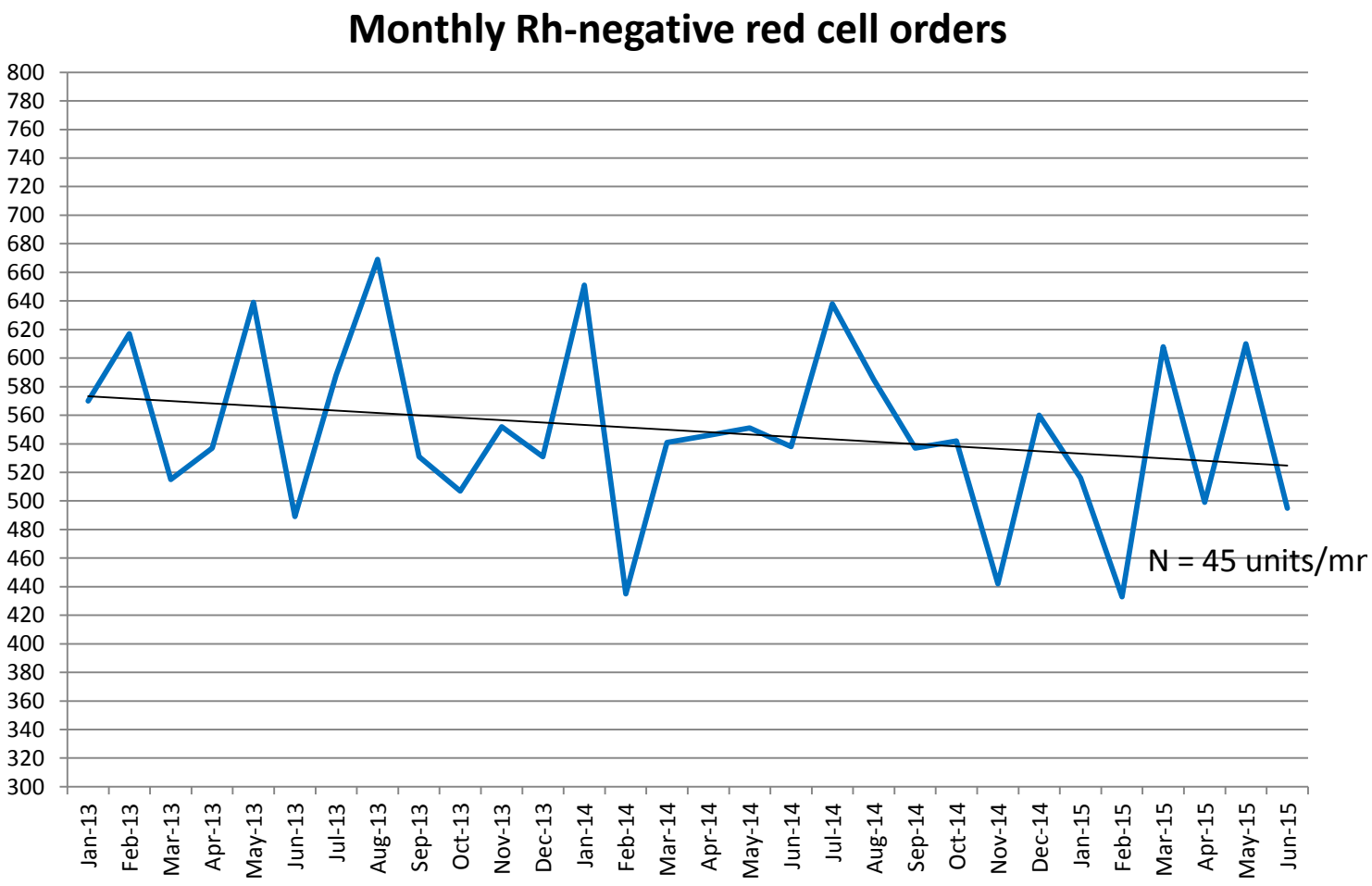
Tailor shipments based on hospital needs (R_0 specialty products to SCD programs)



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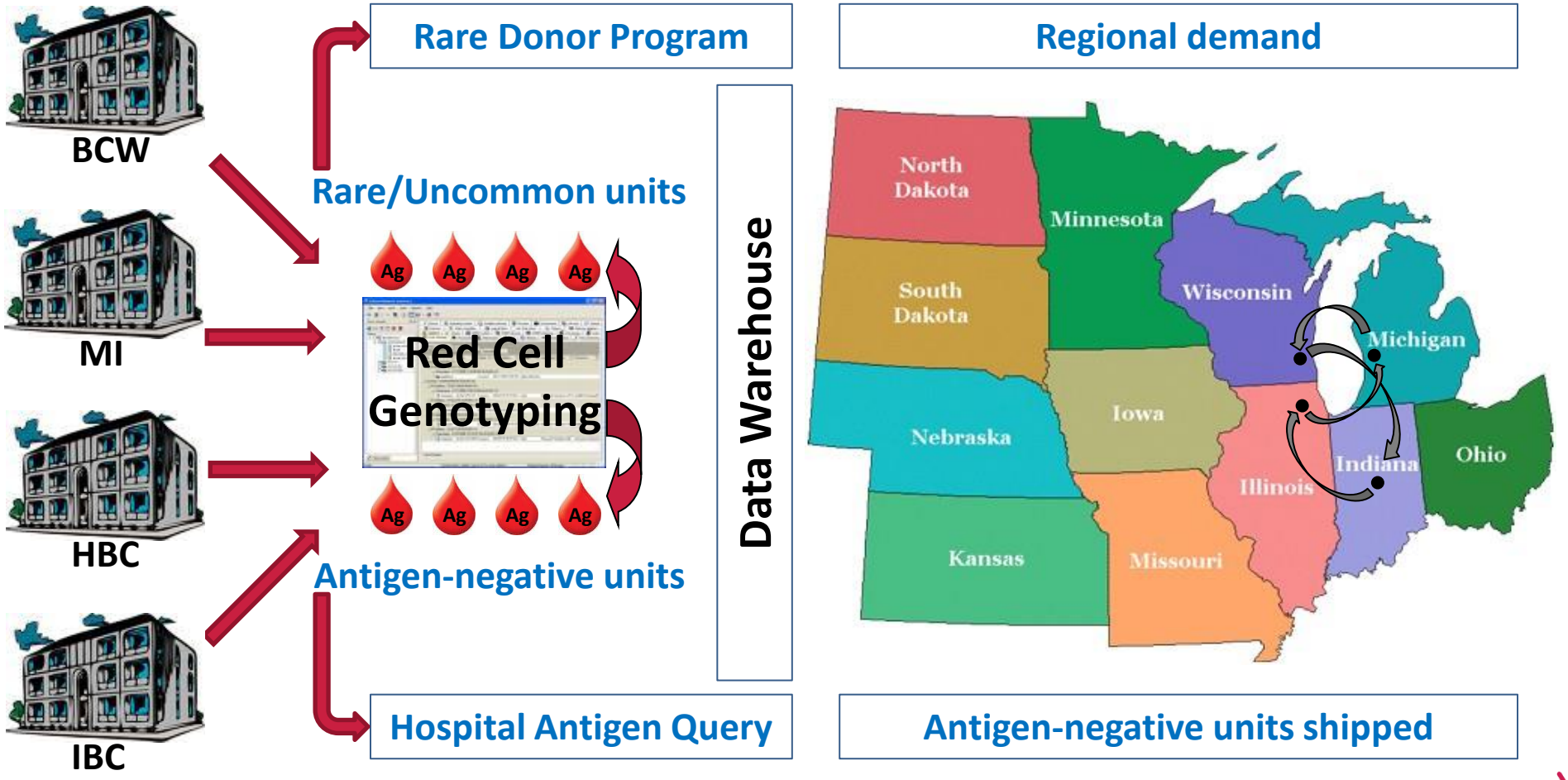
Regional Inventory Management



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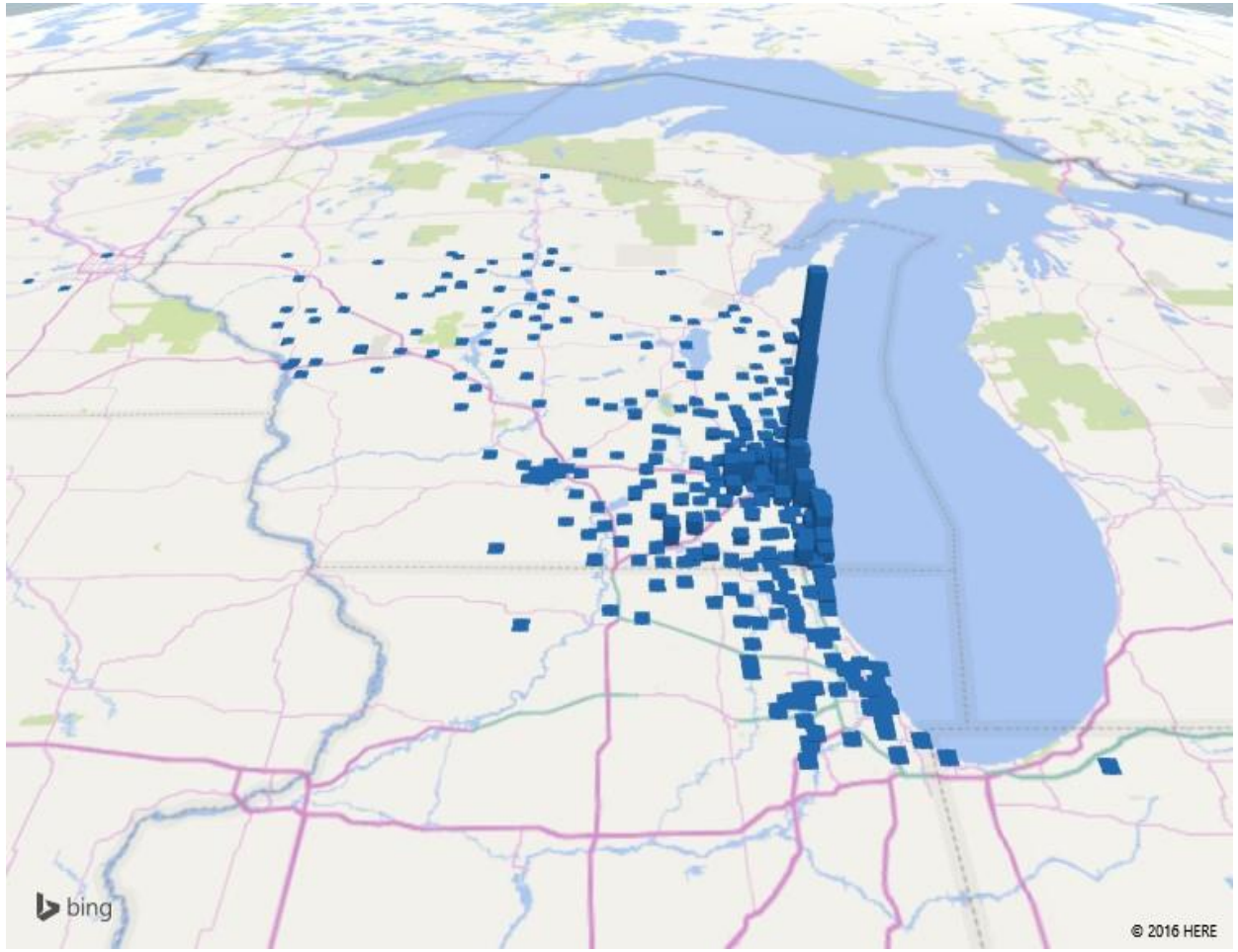
Donor geography – specific nucleotide constellation (Ro)



Identify allele ‘hotspots’ by correlating genotype to address

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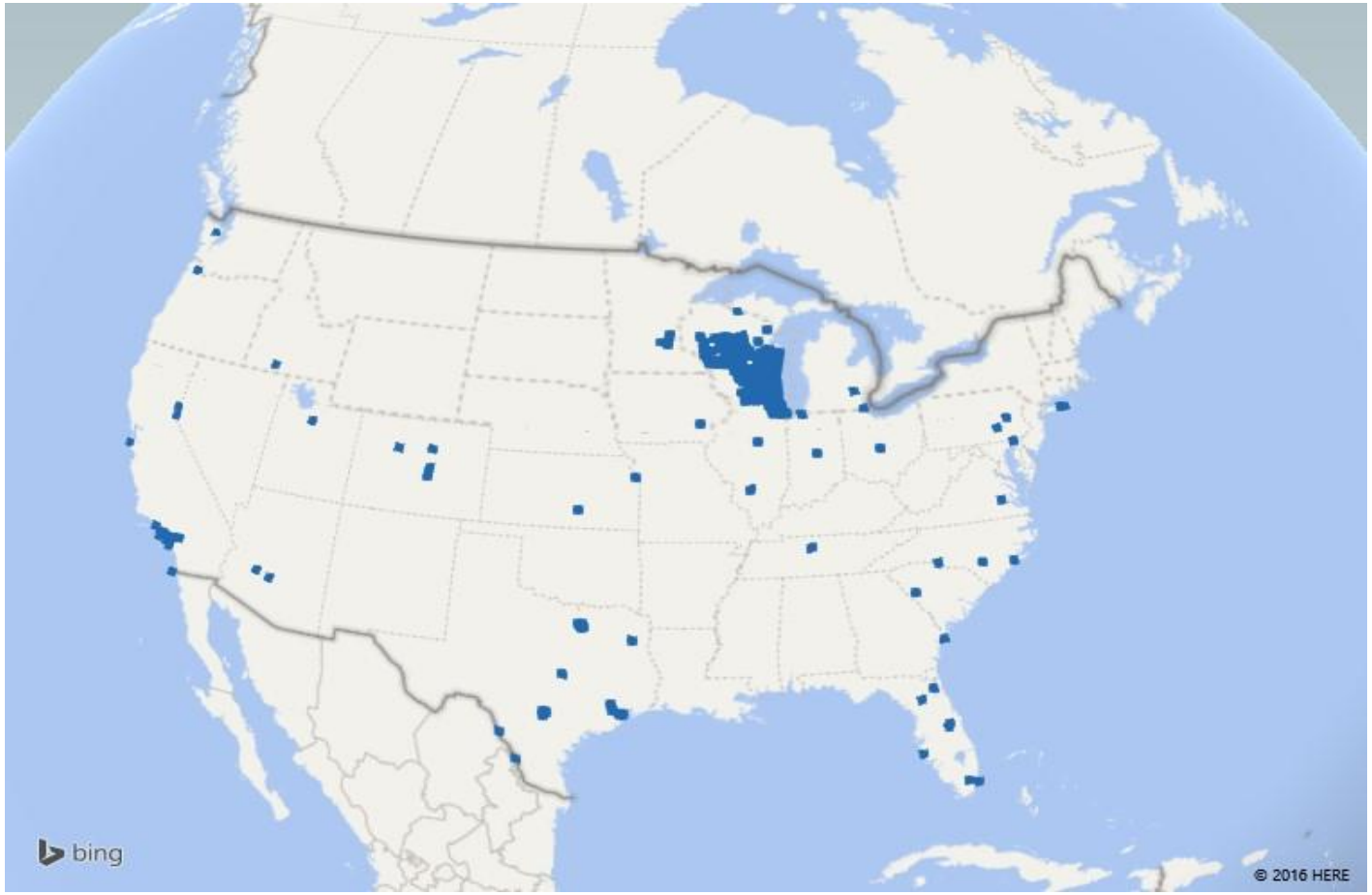
Genotyped donors by ‘zipcode’ address



Movement of genotyped donors benefits the transfusion recipient

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Donor antigen list

Predicted Phenotypes

C/c, E/e	Fy^a/Fy^b	U, Uvar	Vel
V, VS, hr^B, hr^S	Fy(a-b-)	Do^a/Do^b	Yt^a/Yt^b
K/k	Jk^a/Jk^b	Hy	Di^a/Di^b
Kp^a/Kp^b	M/N	Jo^a	Co^a/Co^b
Js^a/Js^b	S/s	Lu^a/Lu^b	Cr^a

RH variants

RHCE 48	RHCE 697	RHCE 818	RHD 410
RHCE 254	RHCE 712	RHCE 916	
RHCE 340	RHCE 733	RHCE 1006	
RHCE 667	RHCE 748	RHCE 1025	

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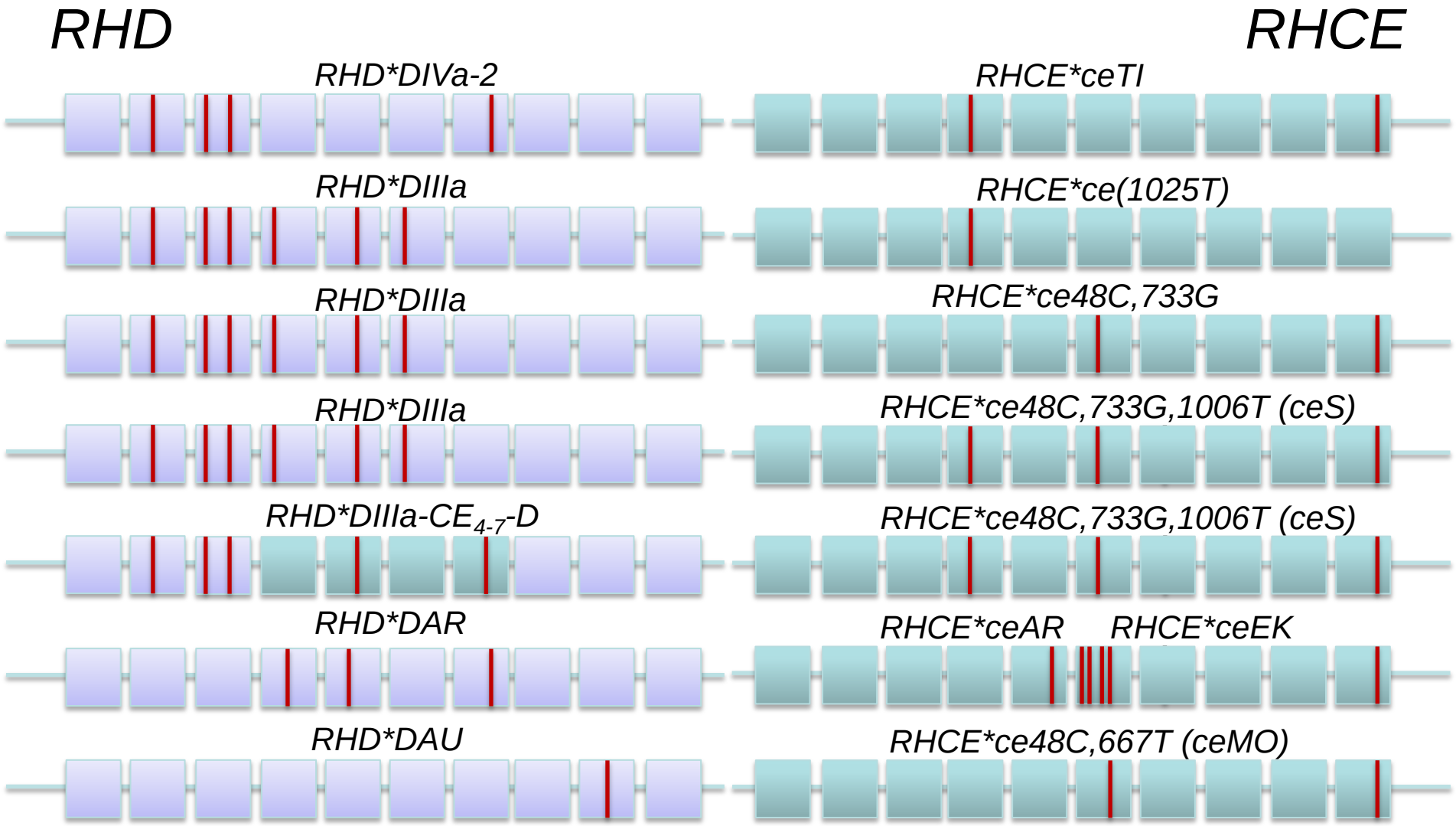
RHD alleles in cis with *RHCE*



The *RH* locus: how many alleles?

Greg Denomme @GregDenomme · 14 Mar 2017

500+ human RHD alleles archived @ ln.is/www.rhesusbase... Who'd a thought so many, and available on ur smartphone! Thx @Squiver for the pic

Data mining

Count of Sample			Intron5			
S/s	S230T	Ethnicity	GG	GT	TT	Grand Total
TC	CC	AA	468	89		557
TC	CC	AS	114			114
TC	CC	CA	4878	2		4880
TC	CC	HI	705	10		715
TC	CC	NA	25			25
TC	CC	OT	141	3		144
TC	CC	(blank)	431	9		440
TC	**CC Total**		**6762**	**113**		**6875**
TC	CT	AA	7			7
TC	CT	CA	2			2
TC	CT	(blank)	1			1
TC	**CT Total**		**10**			**10**
TC Total			6772	113		6885
TT	**CC**	**AA**	**81**	**12**	**7**	**100**
TT	CC	AS	21			21
TT	CC	CA	1201			1201
TT	CC	HI	171	1		172
TT	CC	NA	6			6
TT	CC	OT	40	3		43
TT	CC	(blank)	98	4		102
TT	CC Total		1618	20	7	1645

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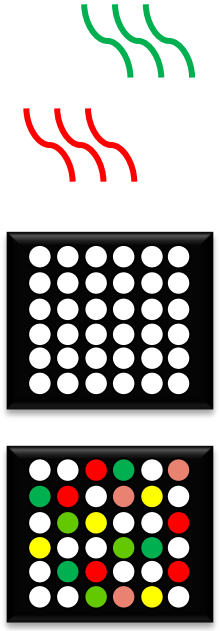
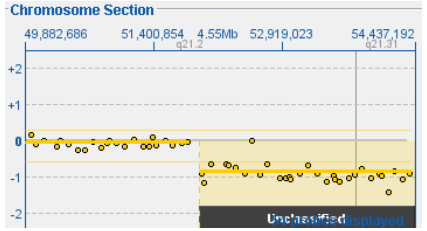
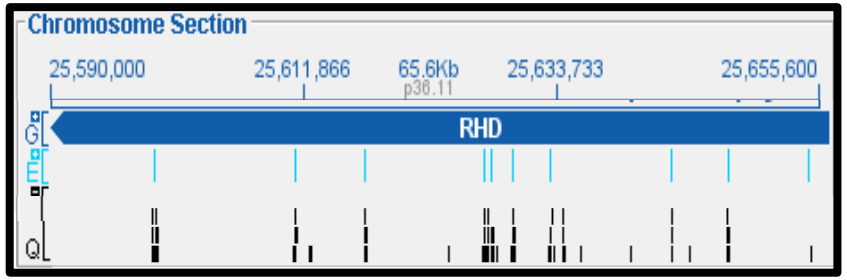
Comparative Genomic Hybridization Arrays

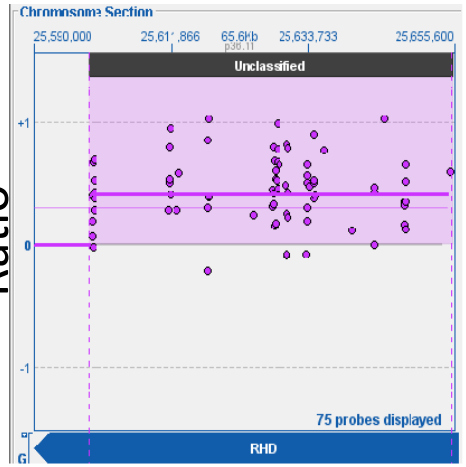


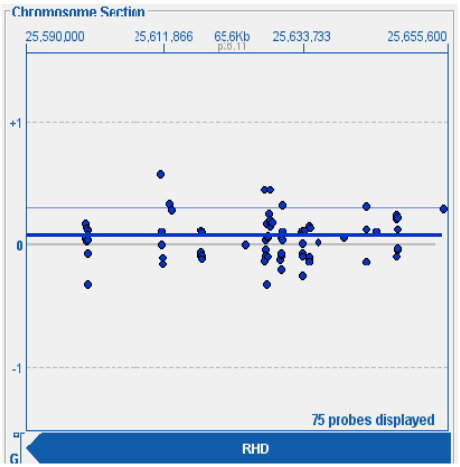


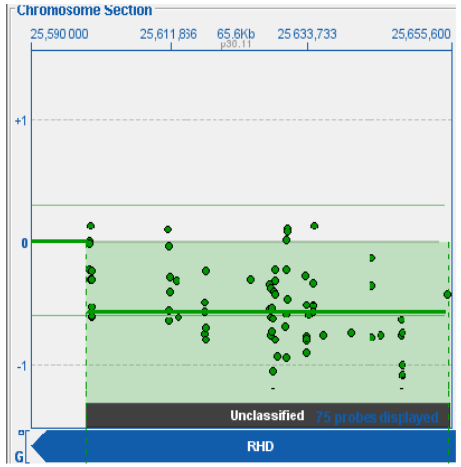


RHD+/RHD+ *RHD+/RHD-* *RHD-/RHD-*

Log Ratio

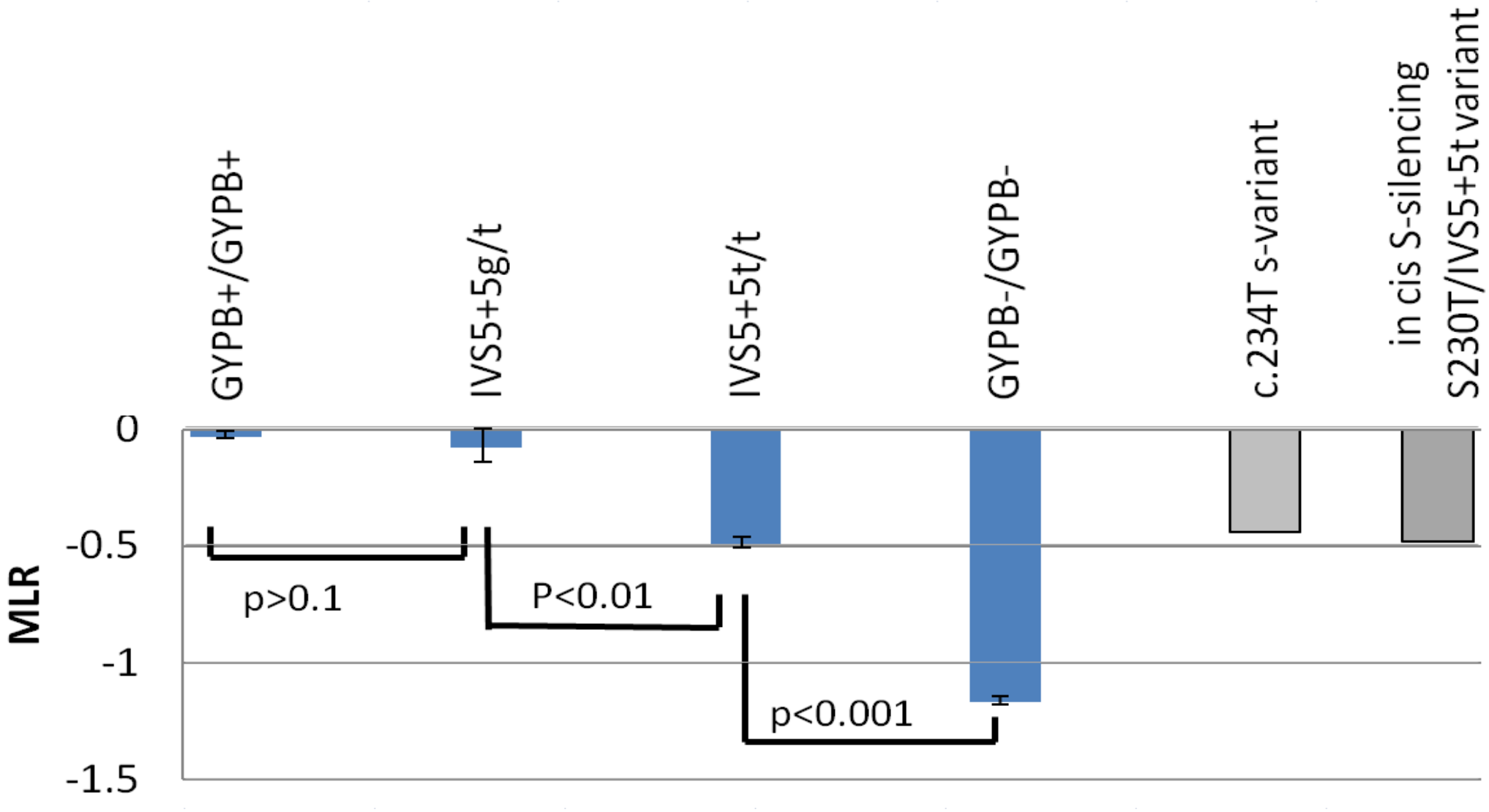






Zygosity	MLR Average	MLR SD	MLR range (± 3SD)	p value
RHD+/RHD+	0.4354	0.019	0.379 – 0.492	P < 0.005
RHD+/RHD-	0.0361	0.062	-0.150 – -0.222	P < 0.001
RHD-/RHD-	-0.502	0.061	-0.686 – -0.319	N/A

Comparative Genomics Hybridization Array



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Accomplishments

Red cell genotyping has transformed the way antigen-negative blood is identified

- electronic handling of mass-scale genotype data (serologic confirmation); made accessible online
- Inventory Management – prevent O Negative 'short-cut'
- Delivery efficiencies – reduce transportation costs
- Inventory sharing by locale / clinics / programs
- Phenotype-Genotype discordances
  - New alleles

Test-of-record will provide efficiencies (label future units without testing)

Expansion of red cell genotyping (R_0 donors), with recruitment strategy

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