Genotyping: Ask The Experts
2018 BCA IRL Meeting

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What does serology offer

Proven method, well integrated in the laboratory

Fast and available

Automated platforms adopted within the field

Abundance of expertise in the field

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What Challenges do Serologists encounter?

Multiple antibodies making it difficult to identify the unknown(s)

Weak reactive antibodies that don't follow the rules

Autoantibody vs. Alloantibody

Limitations of the methods: Recent Transfusion/Positive DAT

Limited by available reagents

TAT: Patient in need of blood: How can we speed up the process

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Case Review: Multiple Antibody Approach

Previous History of anti-E

Genotype was performed on prior admission

Predicted Phenotype:

- C-E-c+e+
- K-k+ Kp(a-b+) Js(a-b+)
- Fy(a+b+)
- Jk(a+b-)

Lu(a-b+)

Do(a-b+)

M+N+S-s+

Lets take a look at the panel with the Genotype information we already know!

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Case Review: Multiple Antibody Approach

	Rh						MNS				LU		P	Lewis		Kell		Duffy		Kidd		PEG
	D	C	E	c	e	f	M	N	S	s	Lu^a	Lu^b	P_1	Le^a	Le^b	K	k	Fy^a	Fy^b	Jk^a	Jk^b	IAT
1) R_1R_1	+	+	0	0	+	0	+	+	+	+	0	+	+	+	0	0	+	+	0	0	+	3
2) rr	0	0	0	+	+	+	+	+	+	0	0	+	0	0	+	+	+	+	+	+	0	4
3) r"r	0	0	+	+	+	+	+	0	+	+	0	+	+	0	+	0	+	0	+	0	+	4
4) R_1R_1	+	+	0	0	+	0	0	+	0	+	0	+	0	+	0	+	0	0	+	+	+	4
5) r'r	0	+	0	+	+	+	+	0	+	0	0	+	0	0	+	+	+	+	+	+	0	4
6) rr	0	0	0	+	+	+	0	+	0	+	0	+	+	0	+	0	+	+	0	0	+	2
7) R_2R_2	+	0	+	+	0	0	+	+	+	+	0	+	+	+	0	0	+	0	+	0	+	4
8) R_1R_2	+	+	+	+	+	0	+	0	+	0	0	+	+	0	+	0	+	+	+	+	0	4
9) R_1r	+	+	0	+	+	+	0	+	0	+	0	+	+	0	+	0	+	+	0	0	+	2
10) R_Or	+	0	0	+	+	+	+	+	+	+	0	+	0	+	0	0	+	0	+	0	+	3
11) R_Or	+	0	0	+	+	+	+	0	0	0	0	+	+	0	+	0	+	0	0	+	+	2
Auto																						0√

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Case Review: Multiple Antibody Approach

Choose Smarter!

Run cells that are…..

- **C+**,E-, S-, K-, Jk(b-)
- C-, **E+,** S-, K-, Jk(b-)
- C-, E-, **S+**, K-, Jk(b-)
- C-, E-, S-, **K+,** Jk(b-)
- C-, E-, S-, K-, **Jk(b+)**
- C-, E-, S-, K-, Jk(b-)

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Case Review: Multiple Antibody Approach

	Rh						MNS				LU		P	Lewis		Kell		Duffy		Kidd		PEG
	D	C	E	c	e	f	M	N	S	s	Lu^a	Lu^b	P_1	Le^a	Le^b	K	k	Fy^a	Fy^b	Jk^a	Jk^b	IAT
1	+	+	0	+	+	+	0	+	0	+	0	+	+	0	+	0	+	+	0	+	0	**3**
2	+	0	+	+	0	0	+	+	0	+	0	+	+	+	0	0	+	0	+	+	0	**4**
3	0	0	0	+	+	+	+	0	+	0	0	+	+	0	+	0	+	+	+	+	0	**2**
4	0	0	0	+	+	+	0	+	0	+	0	+	+	0	+	+	+	+	0	+	0	**4**
5	0	0	0	+	+	+	+	+	0	+	0	+	0	+	0	0	+	0	+	0	+	**2**
6	+	0	0	+	+	+	+	0	0	0	0	+	+	0	+	0	+	0	0	+	0	**0√**
Auto																						**0√**

Run some more select cells and we are done!

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Case Review: Multiple Antibody Approach

Anti-E, anti-C, anti-K, anti-S and anti-Jk^b

Ensures much faster TAT on results, and provision of blood

Negates the need for specialized testing (efficiency):

- enzyme/chemical treatment of cells
- cell separations
- adsorptions
- trial and error method of selecting cells.

Effective approach for chronically transfused patients

Samples previously sent to a Reference Lab may now be solved in the transfusion service.

By having the genotyping results available, we are able to work smarter and speed up the antibody identification process dramatically!

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Case Review: The Unidentifie

History of anti-E, anti-K and anti-Fy^{a}

Has been transfused since last workup, but not within the past 3 months

Unable to locate compatible blood for cardiac surgery

Request is for 8 units of red cells.

Surgery has been postponed until compatible blood can be provided.

STAT

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Case Review: The Unidentified

	Rh						MNS				LU		P	Lewis		Kell		Duffy		Kidd		PEG
	D	C	E	c	e	f	M	N	S	s	Lu^a	Lu^b	P_1	Le^a	Le^b	K	k	Fy^a	Fy^b	Jk^a	Jk^b	IAT
1	+	0	0	+	+	+	+	+	+	+	0	+	0	+	0	0	+	0	+	0	+	1
2	+	0	0	+	+	+	+	0	0	0	0	+	+	0	+	0	+	0	0	+	+	1
3	+	0	0	+	+	+	+	+	+	+	0	+	0	+	0	0	+	0	+	0	+	0√
4	+	0	0	+	+	+	+	0	0	0	0	+	+	0	+	0	+	0	0	+	+	W
Auto																						0√

	Rh						MNS				LU		P	Lewis		Kell		Duffy		Kidd		Ficin
	D	C	E	c	e	f	M	N	S	s	Lu^a	Lu^b	P_1	Le^a	Le^b	K	k	Fy^a	Fy^b	Jk^a	Jk^b	IAT
1	+	0	0	+	+	+	+	+	+	+	0	+	0	+	0	0	+	0	+	0	+	2
2	+	0	0	+	+	+	+	0	0	0	0	+	+	0	+	0	+	0	0	+	+	2
3	+	0	0	+	+	+	+	+	+	+	0	+	0	+	0	0	+	0	+	0	+	0√
4	+	0	0	+	+	+	+	0	0	0	0	+	+	0	+	0	+	0	0	+	+	1
Auto																						0√

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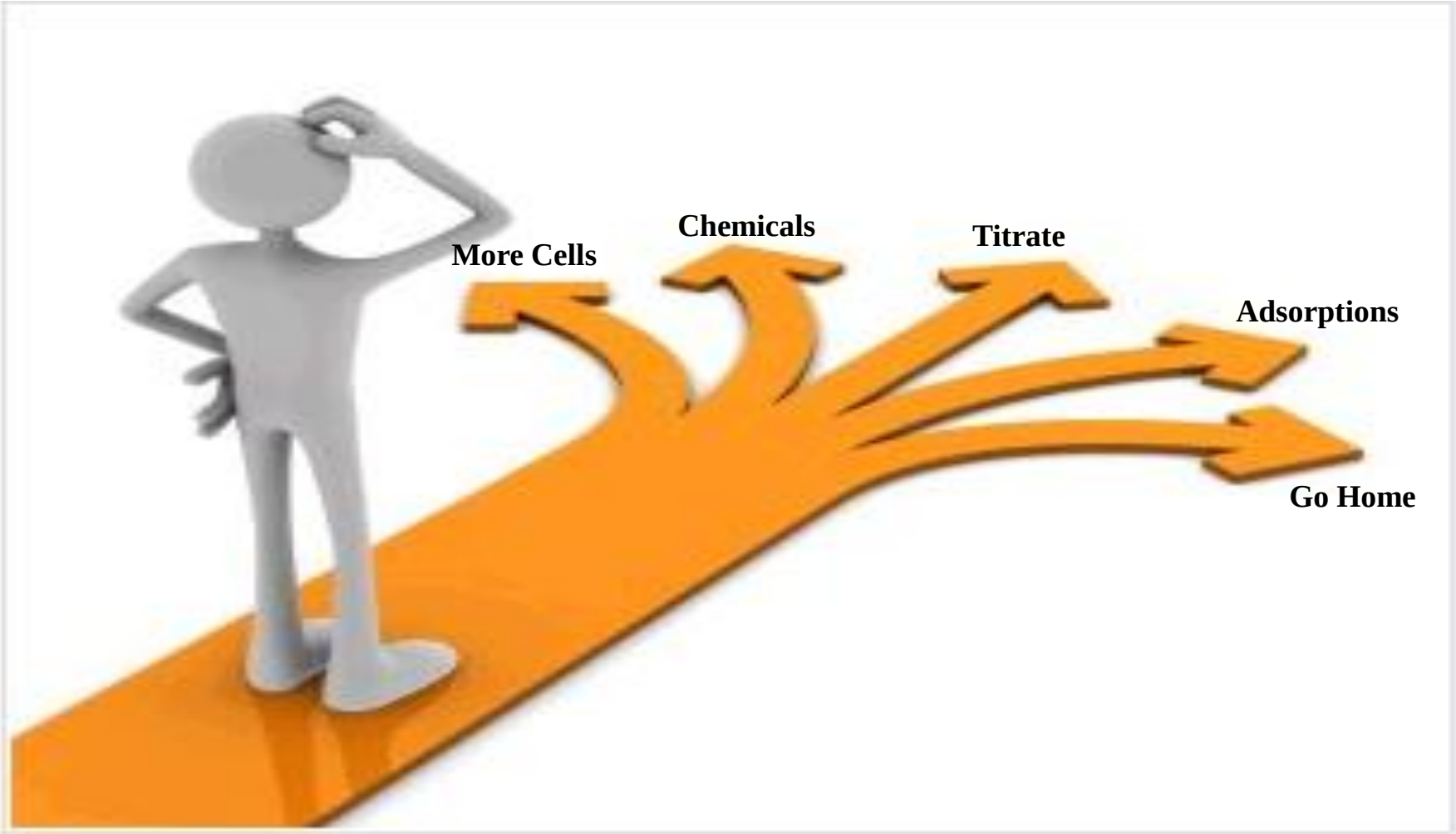
Case Review: The Unidentified



Case Review: The Unidentified

Time for STAT Genotyping!

Predicted Phenotype:

- C+E-c+e+
- K-k+ Kp(a-b+) Js(a-b+)
- Fy(a-b+)
- Jk(a+b+)
- M+N-S+s+
- Lu(a-b+)
- Do(a+b-)

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Case Review: The Unidentified

The Ah-Ha Moment……

She's already made the E, K, and Fy^a

Maybe an N? But the pattern doesn't fit.

What about that Do^b?

- Dombrock status not often on our panel sheets
- Often overlooked specificity

Predicted Phenotype:

C+E-c+e+
K-k+ Kp(a-b+) Js(a-b+)
Fy(a-b+)
Jk(a+b+)
M+N-S+s+
Lu(a-b+)
Do(a+b-)

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Case Review: The Unidentified

	Rh					MNS				Lutheran		P	Lewis		Kell		Duffy		Kidd		Dombrock		PEG
	D	C	E	c	e	M	N	S	s	Lu^a	Lu^b	P_1	Le^a	Le^b	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Doa	Dob	IAT
1	+	+	0	0	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	+	0	+	1
2	+	+	0	0	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	+	0	+	1
3	+	+	0	0	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	+	0	+	1
4	+	+	0	0	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	+	+	0	0√
5	+	+	0	0	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	0	+	0	0√
6	+	+	0	0	+	+	0	0	+	0	+	+	0	+	0	+	0	+	+	+	+	0	0√
Pt																							0√

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Case Review: The Unidentifie

Genotyping was able to give us information when serology could not.

- Do^b antisera is very difficult to come by
- Antisera not commercially available

Difficult to find Do(b-) blood.

Not necessarily due to the antigen frequency (83%)

Red cell Genotyping necessary to identify many Do(b-) donors

8 units of E-, K-, Fy(a-), Do(b-) blood were in inventory and sent to the requesting hospital.

Turn Around Time for genotyping results are critical in these scenarios.

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Case Review

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Case Review: Auto or Allo?

Referred to our IRL for investigation of Warm Autoimmune Hemolytic Anemia, and Red Cell Genotyping

- Patient recently transfused with 4 units of red blood cells
- Hospital policy to obtain a red cell genotype on patients with WAIHA
- Hospital suspects a warm autoantibody present in the patient's serum.
  - Eluate positive with all cells tested
  - No history of alloantibodies.

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Case Review: Auto or Allo?

ABO/RH						
Front Type				Reverse Type		
Anti-A	Anti-B	Anti-A,B	Anti-D	A_1	A_2	B
4+	0	4+	4+	0	0	4+

DAT				
Poly	IgG	Anti-C3b,-C3d	Anti-C3d	Control
3+	3+	1	1	0
3+		2	2	0

Eluate: 3+ with all cells tested

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Case Review: Auto or Allo?

	Rh						MNS				Lu		P	Lewis		Kell		Duffy		Kidd		Saline		
	D	C	E	c	e	f	M	N	S	s	Lu^a	Lu^b	P_1	Le^a	Le^b	K	k	Fy^a	Fy^b	Jk^a	Jk^b	IS	37C	IAT
1) R_1R_1	+	+	0	0	+	0	+	+	+	+	0	+	0	+	0	0	+	0	+	0	+	0	0	3
2) rr	0	0	0	+	+	+	+	0	+	0	0	+	$+^w$	0	+	+	0	+	+	+	0	0	0	4
3) r"r"	0	0	+	+	0	0	0	+	0	+	0	+	$+^s$	0	+	+	+	+	0	0	+	0	0	4
4) R_1R_1	+	+	0	0	+	0	+	0	+	+	0	+	0	0	0	0	+	+	+	+	+	0	0	3
5) r"r	0	0	+	+	+	+	+	+	0	+	0	+	0	0	+	0	+	0	+	0	+	0	0	3
6) r'r'	0	+	0	0	+	0	+	+	0	+	0	+	+	0	+	+	+	+	+	+	+	0	0	4
7) R_2R_2	+	0	+	+	0	0	+	+	+	0	+	+	+	0	+	0	+	+	0	+	0	0	0	3
AC																						0	0	3

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Case Review: Auto or Allo?

Ficin: 4+ with all cells tested

Alloadsorptions performed on the patient serum since recently transfused

- Anti-K identified in the alloadsorbed serum.

Warm Auto with underlying anti-K

Now to result the genotyping

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Case Review: Auto or Allo?

Red Cell Genotyping Results:

Predicted Phenotype:

- C+E-c+e+
- K-k+ Kp(a+b-) Js(a-b+)
- Fy(a+b-)
- Jk(a-b+)
- M+N-S+s-
- Lu(a-b+)
- Do(a+b+)

Hold Everything!

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Case Review: Auto or Allo?

Summary:

- Anti-Kp^b identified in the patient's serum and eluate.
- Anti-K also in the serum.
- Transfusion reaction! Not WAIHA
- Alloadsorptions always run the risk of removing an antibody to a high frequency antigen.
  - Red Cell Genotyping can reduce this risk

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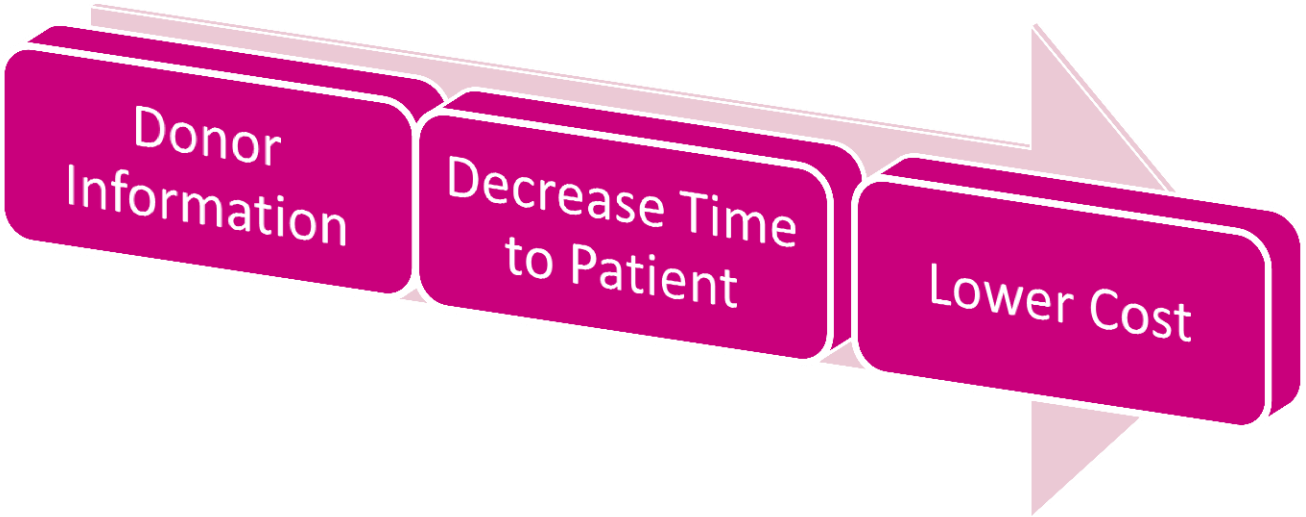
Identifying Donors using Serology and Red Cell Genotyping

Find a **cost effective** means to **obtain** and **share information** on donors to the transfusion services we serve, in order to **decrease their cost** while providing the product in the most **time efficient** manner possible.



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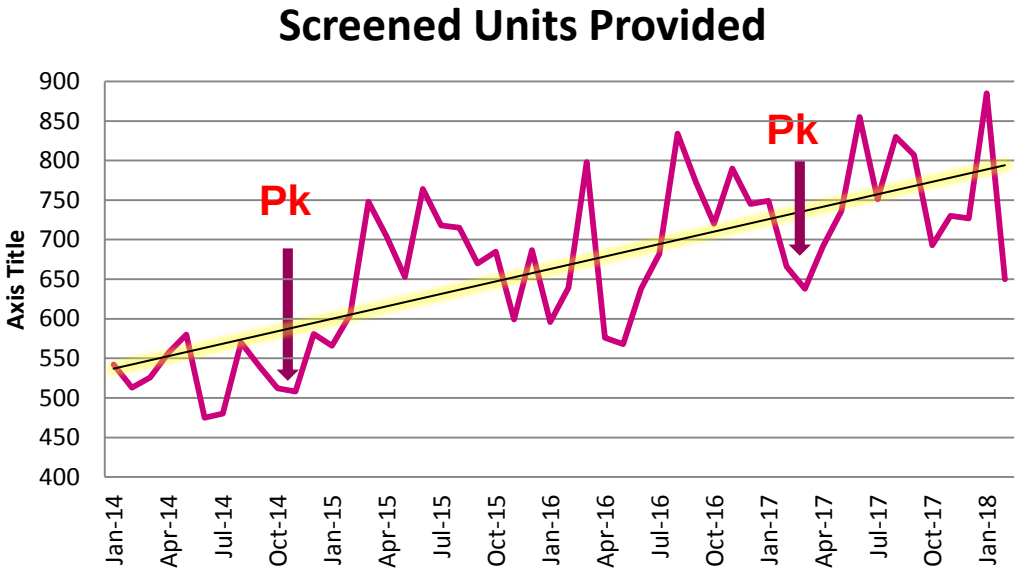
Serologic Screening



Pk

Use diluted Antisera and still get acceptable results.

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Serologic Screening Results

May 2017 Through Dec 2017					
	Wisconsin	Illinois	Indiana	Michigan	Total
Total Pk Screened	82380	52297	60164	56941	**251782**
Ro	667	1331	1282	962	4242
R2 (e-)	545	850	979	844	3218
Opos, R1 (c-) 10%	675	438	589	257	1959
AA	233	764	452	454	1903
(O/A)Rh Negative	908	1618	1553	501	4580
Other	0	7	1	2	10
Total Genotyped	3028	5008	4856	3020	**15912**

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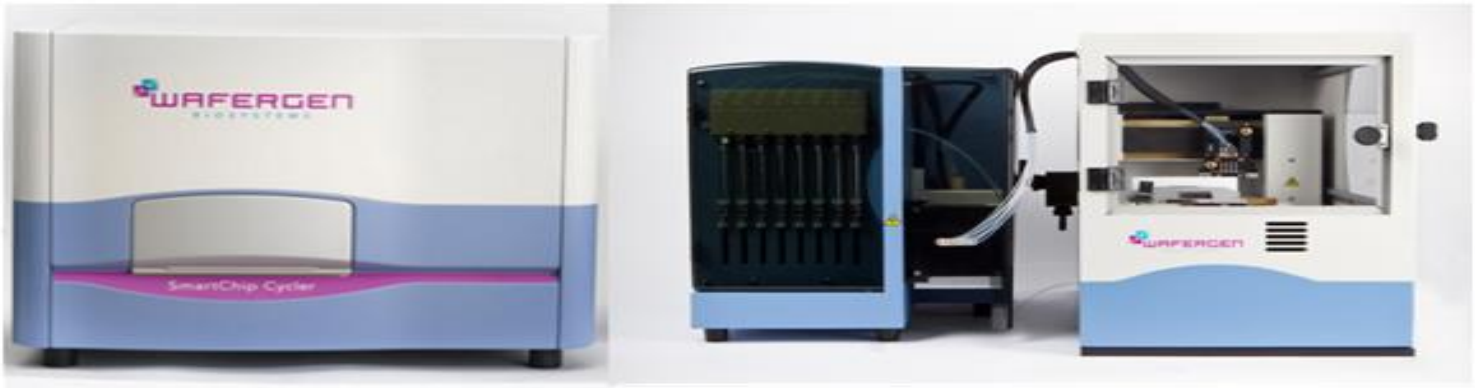
Mass Scale Red Cell Genotyping

WAFERGEN BIOSYSTEMS



SmartChip Genotyping

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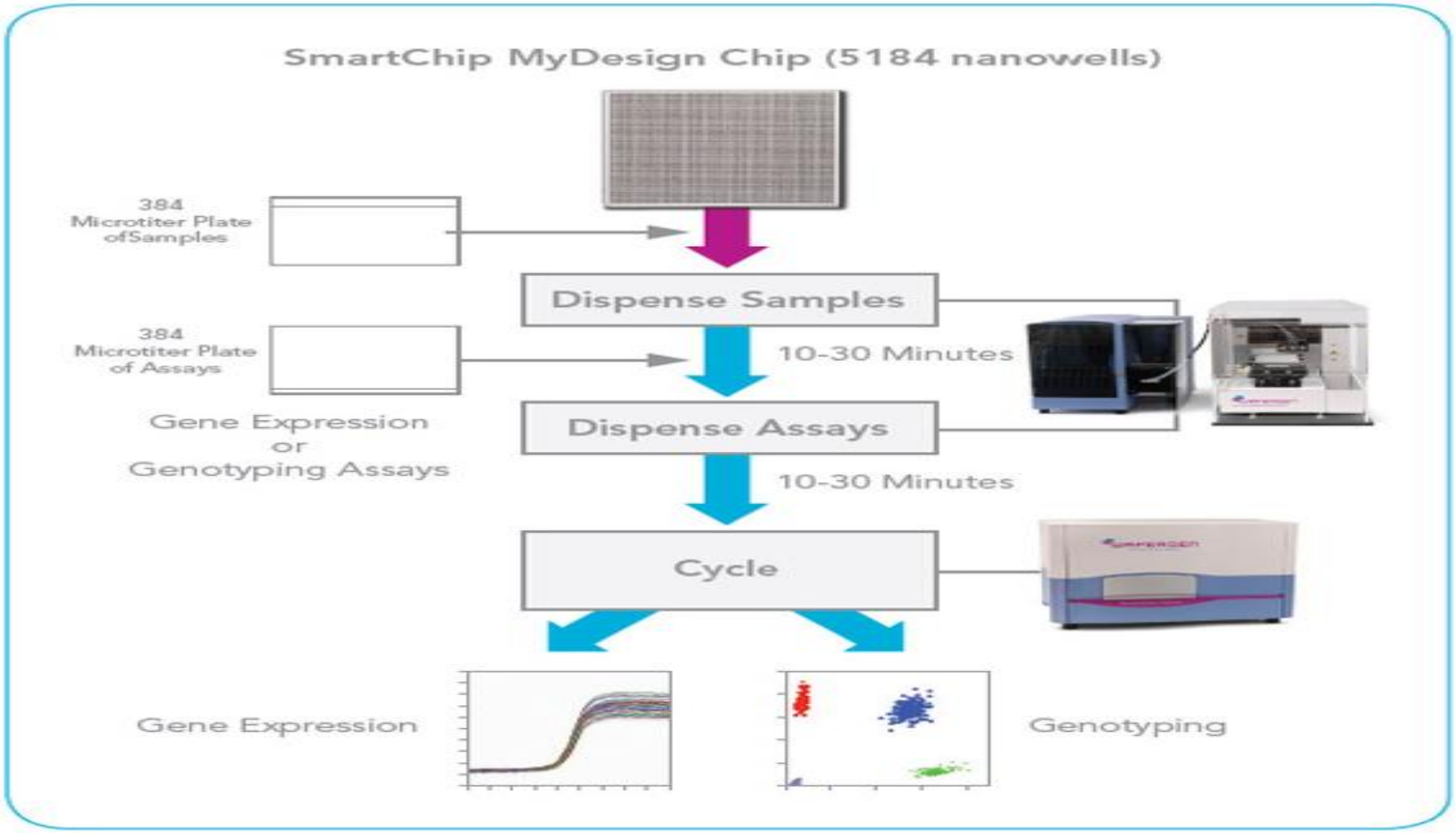
Mass Scale Red Cell Genotyping



Donor Genotyping Results

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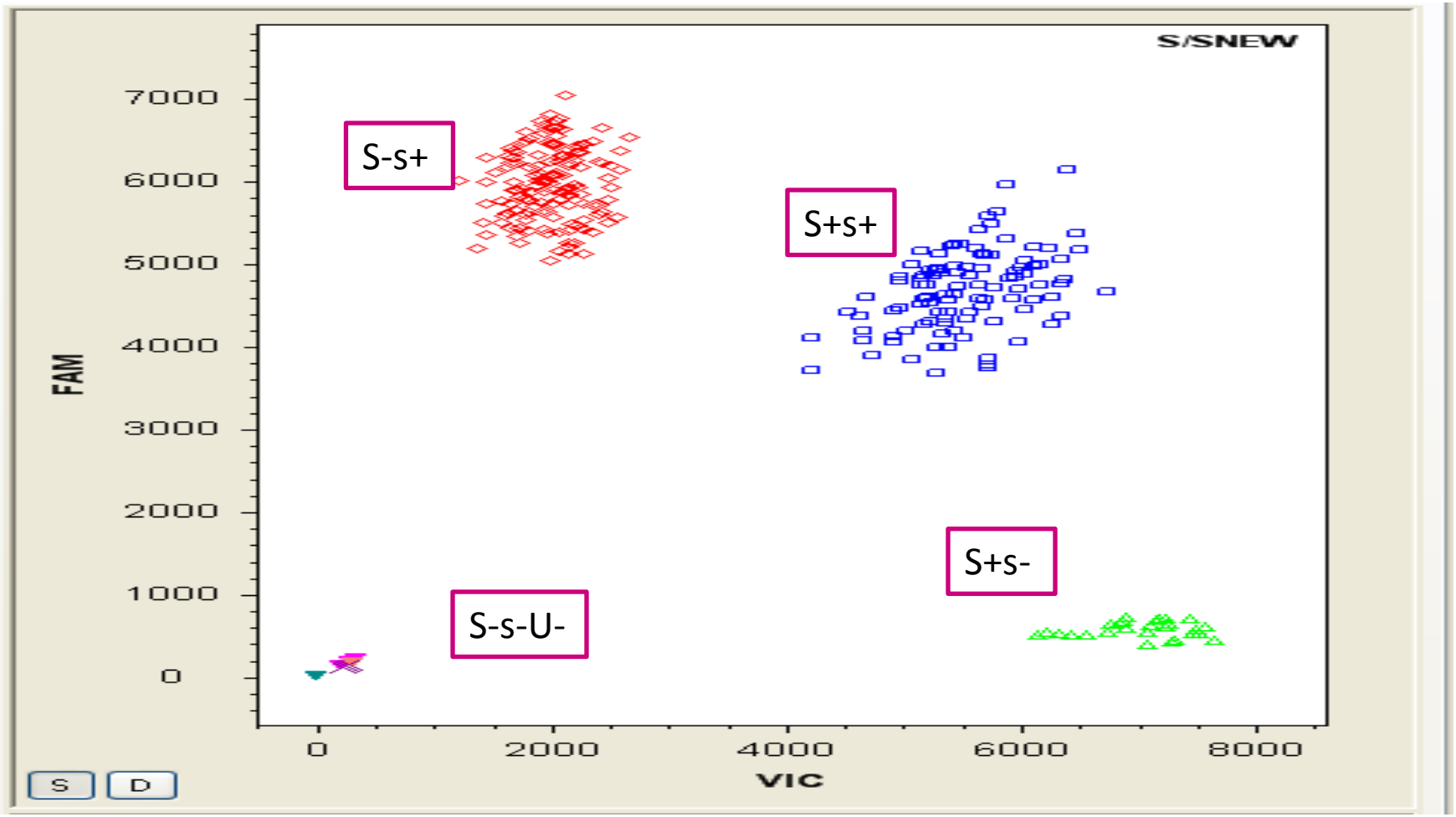

RCG Antigen List

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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

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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Unit sharing among Versiti

National ARDP

Rare/Uncommon units

Ag Ag Ag Ag

Ag Ag Ag Ag

Ag-neg units

Antigen-negative units visible across centers

Data Warehouse

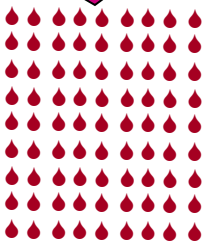
Illinois

Michigan

Indiana

Wisconsin

How Information Improves Efficiency



Historically Typed
Donor base
Molecular/Serology

Blood Center IRLs

Confirmed/labeled

Confirmed/labeled

Hospital Transfusion Service

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Other Applications

Weak D/Partial D Typings

AABB and CAP Joint Statement on Phasing-In *RHD* Genotyping for Pregnant Women and Other Females of Childbearing Potential with a Serologic Weak D Phenotype

- *RHD* genotyping is recommended whenever a weak D phenotype is detected by routine Rh blood typing of pregnant women and other females of childbearing potential.

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Other Applications

Molecular Confirmation of Antigens

- RhCE Variants
- True U Negative vs. U Variant

“Genotype matching” patients

- Cases of Warm Auto, HTLA, Anti-CD38, unidentified antibodies, STAT, chronically transfused

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What does molecular offer

- Ability to determine a predicted phenotype from a patient sample regardless of transfusion
- Ability to determine a predicted phenotype from a patient sample with a positive DAT
- Gather a large amount of information on a patient or donor when antisera cannot
- Allows us to be more proactive rather than reactive to antibody identification workups
- Can help decrease TAT for patient antibody identification problems and procurement of the appropriate products

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What challenges does molecular face

Genotype does not always reflect phenotype!!!

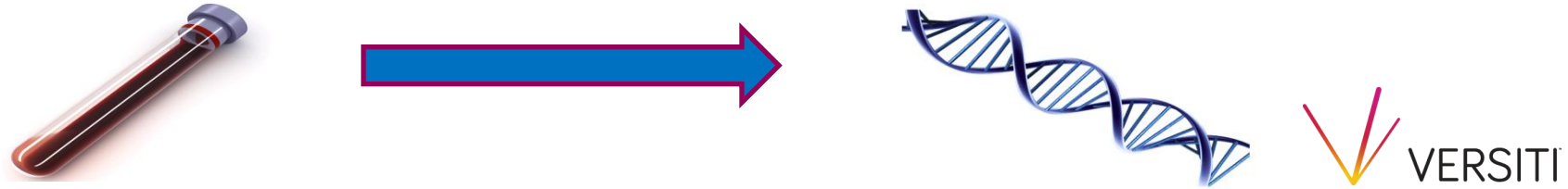
- Assays may not detect rare nucleotide changes leading to altered or partial antigen expression.
- Null phenotypes may not be detected by some methods.

Availability/Cost:

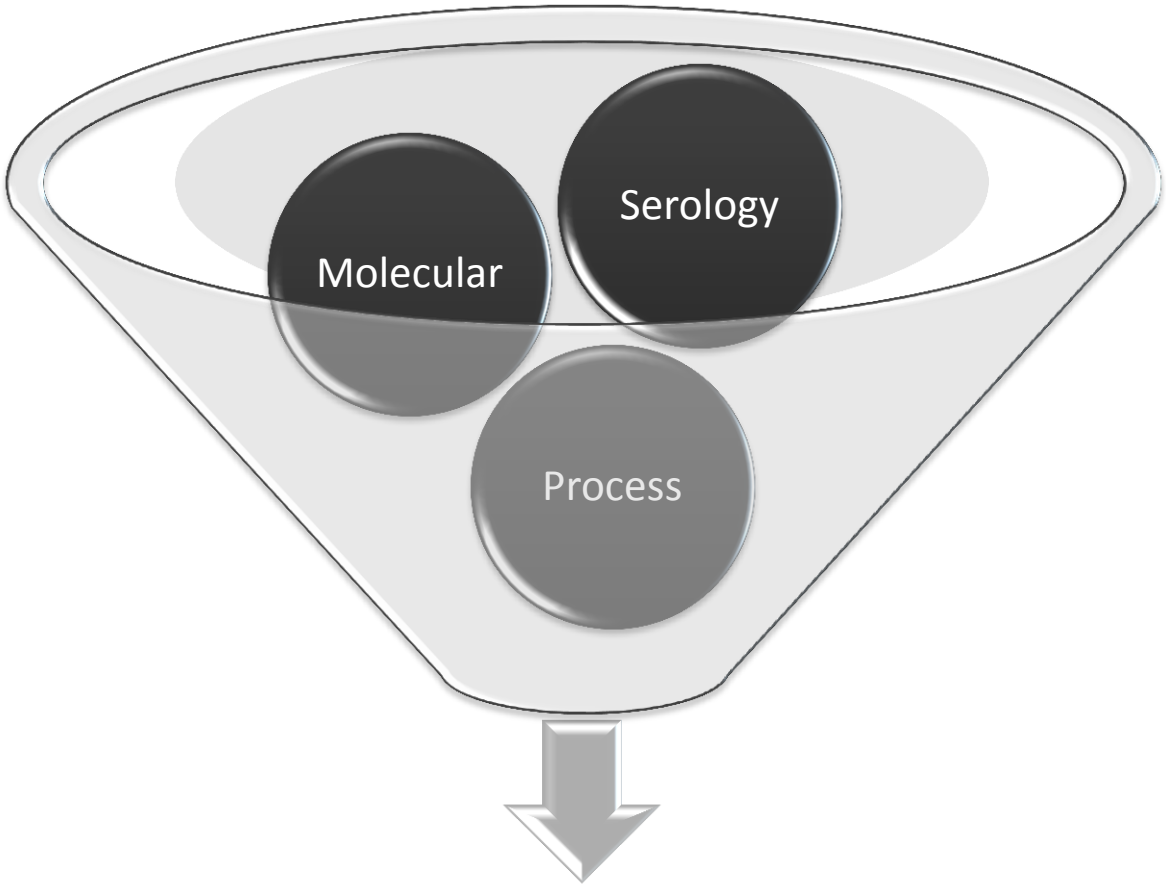
- Not in many Transfusion Services, not in all Blood Centers
- Becoming more affordable

Requires DNA isolation

- Longer TAT, special techniques, special workflow



In summary



The right product….faster

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