Combining Serology and Genotyping to Improve Patient Outcomes: Case Studies

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Objectives

1. List limitations of common serological techniques.

2. List limitations of common genotyping techniques.

3. Recognize test data that may help resolve complex investigations.

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Limitations of Serological Techniques

- Recent transfusion(s)
- Warm Autoantibodies
- Unavailability of reagents
- Antigen variants
- MoAb Therapy

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Limitations of Serological Techniques

Recent transfusion

- Antigen typing
  - Mixed-field reactivity
- Positive DAT and/or Autocontrol
  - Warm Autoantibody?
  - Alloantibody?
- Hemodilution

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Limitations of Serological Techniques

Warm Autoantibodies

- Complicates antigen typing
  - False-positive reactions with IAT reagents
    - Chloroquine diphosphate
    - EDTA/glycine-acid (EGA)
- IAT Interference
  - Panreactivity

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Limitations of Serological Techniques

Unavailability of Reagents

- Antisera for high prevalence antigens
- Antisera for low prevalence antigens
- Antisera for “non-common” blood group antigens

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Limitations of Serological Techniques

Antigen variants

- Discrepant reactivity with reagent antisera
- Partial antigens
  - RhD
  - RhCE
- Hybrid antigens
  - “False-positive” reactions
    - Anti-M

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Limitations of Serological Techniques

MoAb Therapy

- Anti-CD38
- Anti-CD47

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Limitations of Common Genotyping Techniques

- Only single nucleotide polymorphisms (SNPs) are interrogated
- Limited to most common SNP variatiants
- Downstream polymorphisms may not be detected
- Cis/Trans arrangement of multiple polymorphisms
- Hybrid alleles
- Allele dropout
  - Polymorphism in primer binding site

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How does this help me?

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Case #1

- 23 Y.O. Male
- Admitted through E.R. after M.V.A.
- MC vs. PU
- Type and Screen ordered
- 4 units pRBCs ordered

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Case #1

- Patient typed as O Positive
- Antibody screen was W-1+ with all cells tested
- Antibody panel was W-1+ with all cells tested
- Autocontrol was weakly positive
- Warm autoantibody was suspected

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Case #1

- Antigen type for Rh, Kell, Kidd, and S/s

  C- c+ $E+^{MF}$ e+ K- Jk^a+ Jk^b- S- s+

- Sent for RBC genotyping as part of WAA work-up
  - Mixed-field reactivity
- RBC genotype report:

  C- c+ E+ e+ K- Jk^a+ Jk^b- S- s+

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Case #1

- Patient had SCD
- Mixed field with anti-E reagent due to recent transfusion
  - Patient received multiple units of E-negative RBCs

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Case #2

- 20 Y.O. Female with SCD
- History of Anti-C, -K, -Fy^{a}, -M, -S, -Le^{a}, and WAA
- Patient routinely transfused with “antigen-negative RBCs”
- Medical director requested consultation work-up due to “vanishing WAA”

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Case #2

	D	C	E	c	e	K	Fy^a	Fy^b	Jk^a	Jk^b	M	N	S	s	PEG	Papain	DTT
1	+	+	0	0	+	0	0	+	+	+	0	+	+	+	W+	1+	W+
2	+	+	0	0	+	+	+	0	+	0	0	+	0	+	W+	1+	W+
3	+	0	+	+	0	0	0	+	0	+	+	+	0	+	W+	1+	0
4	0	+	0	+	+	0	0	+	+	+	+	+	+	0	W+	1+	W+
5	0	0	+	+	+	0	+	0	0	+	+	+	0	+	W+	1+	0
6	+	0	0	+	+	0	0	0	+	0	+	+	+	+	0	0	0
7	0	0	0	+	+	+	+	+	+	+	+	0	+	0	W+	W+	0
8	0	0	0	+	+	+	0	+	0	+	+	0	0	+	W+	1+	0
9	0	0	0	+	+	0	+	+	+	0	+	+	+	+	W+	1+	0
10	0	0	0	+	+	0	+	0	+	+	+	+	+	0	0	0	0
AC															W+	W+	0

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Case #2

- Antigen resistant to papain treatment
- Antigen sensitive to 0.2M DTT
- Kell, Lutheran, Knops, Dombrock, LW, Cartwright (maybe)
- Trypsin treat RBCs?

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Case #2

System	Interrogated Alleles	Sample Genotype	Predicted Phenotype	
Rh	*RHCE*ce* *RHCE*Ce* *RHCE*cE* *RHCE*CE* *RHCE*CeCW* *RHCE*ceCW* *RHCE*CECW* *RHCE*ceAR* *RHCE*CeFV* *RHCE*CeVG* *RHCE*cEFM* *RHCE*ce[712G]* *RHCE*ce[733G]* *RHCE*ce[733G,1006T]* *RHCE*CE-D(2,5,7)-CE* *RHCE*cE[697G,712G,733G]* *RHD*Ys-RHCE*ce[733G,1006T]*	*RHCE*ce* *RHD*Ys-RHCE*ce[733G,1006T]*	C (RH:2)	+(29)
			E (RH:3)	0
			c (RH:4)	+
			e (RH:5)	+
			CW (RH:8)	0
			V (RH:10)	0
			hrS (RH:19)	+
			VS (RH:20)	+
			hrB (RH:31)	+
Kell	*KEL*K* *KEL*k* *KEL*KPA* *KEL*JSA*	*KEL*k*	K (KEL:1)	0
			k (KEL:2)	+
			Kpa (KEL:3)	0
			Kpb (KEL:4)	+
			Jsa (KEL:6)	0
			Jsb (KEL:7)	+
Kidd	*JK*A* *JK*B* *JK*B(IVS5-1A)* *JK*B(-871C)*	*JK*A* *JK*B*	Jka (JK:1)	+
			Jkb (JK:2)	+
Duffy	*FY*A* *FY*B* *FY*B(-67C)* *FY*B[265T]*	*FY*B* *FY*B(-67C)*	Fya (FY:1)	0
			Fyb (FY:2)	+
MNS	*GYPA*M* *GYPA*N* *GYPB*S* *GYPB*s* *GYPB*S(IVS5+5T)* *GYPB*S(230T)* *GYPB*deletion* *GYPB*Mur*	*GYPA*N* *GYPB*s*	M (MNS:1)	0
			N (MNS:2)	+
			S (MNS:3)	0
			s (MNS:4)	+
			U (MNS:5)	+
			Mia (MNS:7)	0
Diego	*DI*A* *DI*B*	*DI*B*	Dia (DI:1)	0
			Dib (DI:2)	+
Dombrock	*DO*A* *DO*B* *DO*B[323T]* *DO*A[350T]*	*DO*B*	Doa (DO:1)	0
			Dob (DO:2)	+
			Hy (DO:4)	+
			Joa (DO:5)	+
Colton	*CO*A* *CO*B*	*CO*A*	Coa (CO:1)	+
			Cob (CO:2)	0
Cartwright	*YT*A* *YT*B*	*YT*A*	Yta (YT:1)	+
			Ytb (YT:2)	0
Lutheran	*LU*A* *LU*B*	*LU*B*	Lua (LU:1)	0
			Lub (LU:2)	+

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Case #2

- Plasma treated with Do^{a} rBGP
- C-positive and K-positive RBCs reactive
- All other RBCs, including autocontrol, were non-reactive
- Anti-C, -K, and -Do^{a} identified

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Case #3

- Customer submitted sample for ABO discrepancy resolution

Anti-A	Anti-B	Anti-D	A_1 Cells	B Cells
0	0	4+	0	0

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Case #3

Sample 2				
Locus	Segment	NT Change	AA Change	Genotype
ABO	Exon 3	c.106G/T	p.36Val/Phe	*ABO*O.01.02,*
ABO	Exon 4	c.188G/A	p.63Arg/His	*ABO*467T,803C,1061delC*
ABO	Exon 4	c.189C/T	p.63Arg/His	
ABO	Exon 5	c.220C/T	p.74Pro/Ser	
ABO	Exon 6	c.261G/delG	p.88Thr/Pro>fs118Ter	
ABO	Exon 6	c.297A/G	p.99Thr/Thr	
ABO	Exon 7	c.467C/T	p.156Pro/Leu	
ABO	Exon 7	c.646T/A	p.216Phe/Ile	
ABO	Exon 7	c.681G/A	p.227Pro/Pro	
ABO	Exon 7	c.771C/T	p.257Pro/Pro	
ABO	Exon 7	c.803G/C	p.268Gly/Ala	
ABO	Exon 7	c.829G/A	p.277Val/Met	
ABO	Exon 7	c.1061C/delC	p.354Pro/Arg>fs376Ter	

- Variants c.467T and c.1061delC are commonly found in alleles encoding an A_2 phenotype
- Variants c.467T and c.803C are found in some alleles encoding a cisAB phenotype (e.g. *ABO*cisAB.01)*
- *ABO*467T,803C,1061delC* allele was unreported

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Case #3

- *ABO*467T,803C,1061delC* allele likely encodes a weak cis-AB-like phenotype
- Second sample received for ABO discrepancy resolution

Anti-A	Anti-B	Anti-D	A_1 Cells	B Cells
0	0	4+	0	W+

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Case #3

Locus	Segment	NT Change	AA Change	Genotype
ABO	Intron 4	IVS4-9T/C	-	*ABO*O.01.56*
ABO	Exon 6	c.261G/delG	p.88Thr/Pro>fs118Ter	*ABO*467T,803C,1061delC*
ABO	Exon 7	c.467C/T	p.156Pro/Leu	
ABO	Exon 7	c.803G/C	p.268Gly/Ala	
ABO	Exon 7	c.1061C/delC	p.354Pro/Arg>fs376Ter	

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Case #3

			IS	RT	4°C	Papain-IS	Papain-RT
Forward ABO	Anti-A	Mono 1	0	0	0	0	W+
		Mono 2	W+	W+	1+	W+	1+
	Anti-B	Mono 1	0	0	0	0	0
		Mono 2	0	0	0	0	0
	Anti-A,B	Mono and Poly	W+	1+	2+	NT	NT
Reverse ABO	A_1 RBCs		0	W+	W+	NT	NT
	A_2 RBCs		0	0	0	NT	NT
	B RBCs		W+	1+	1+	NT	NT

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Case #3

- Variants c.467T and c.1061delC are commonly found in alleles encoding an A_2 phenotype
- Variants c.467T and c.803C are found in some alleles encoding a cisAB phenotype (e.g. *ABO*cisAB.01)*
- *ABO*467T,803C,1061delC* allele likely encodes a weak cis-AB-like phenotype
- Unable to determine if different serological results was due to different testing methodologies
- Pending additional investigation

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Case #4

- Patient received anti-CD38 therapy for multiple myeloma
- Per protocol, customer submits sample for blood group genotyping
- Blood group genotyping results reported to customer
- Predicted RhCE phenotype: C+E+c+e-
- Customer emails to report genotype/phenotype discrepancy

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Case #4

- Customer performed RhCE phenotype
    - Patient typed as C+E+c+e+
- *RHCE* gene sequencing performed to resolve discrepancy
- Sequencing data reproduced array prediction
    - Predicted phenotype was C+E+c+e-
- Sequencing results reported to customer

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Case #4

- Customer emails to complain about genotype/phenotype discrepancy
- Additional samples are submitted for repeat testing
- One sample is sent for RhCE antigen typing
- Patient’s RhCE phenotype was C+E+c+e+
- e antigen typing was tested with three different monoclonal reagents and one polyclonal source

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Case #4

- *RHCE* exon 5 PCR repeated with alternate primers
- Predicted phenotype matched serological typing
- Polymorphism discovered in the primer binding site

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Conclusion

- Be familiar with reagent performance characteristics
- Be familiar with assay/reagent limitations
- There is no “One Size Fits All” solution
- Synergy is key
- Sometimes there is no clear cut answer

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Questions?

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Thank you!

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