Molecular Testing: What can it do for the Blood Bank

Virginia Hare, MT(ASCP) SBB

American Red Cross Southern Region Reference Laboratory

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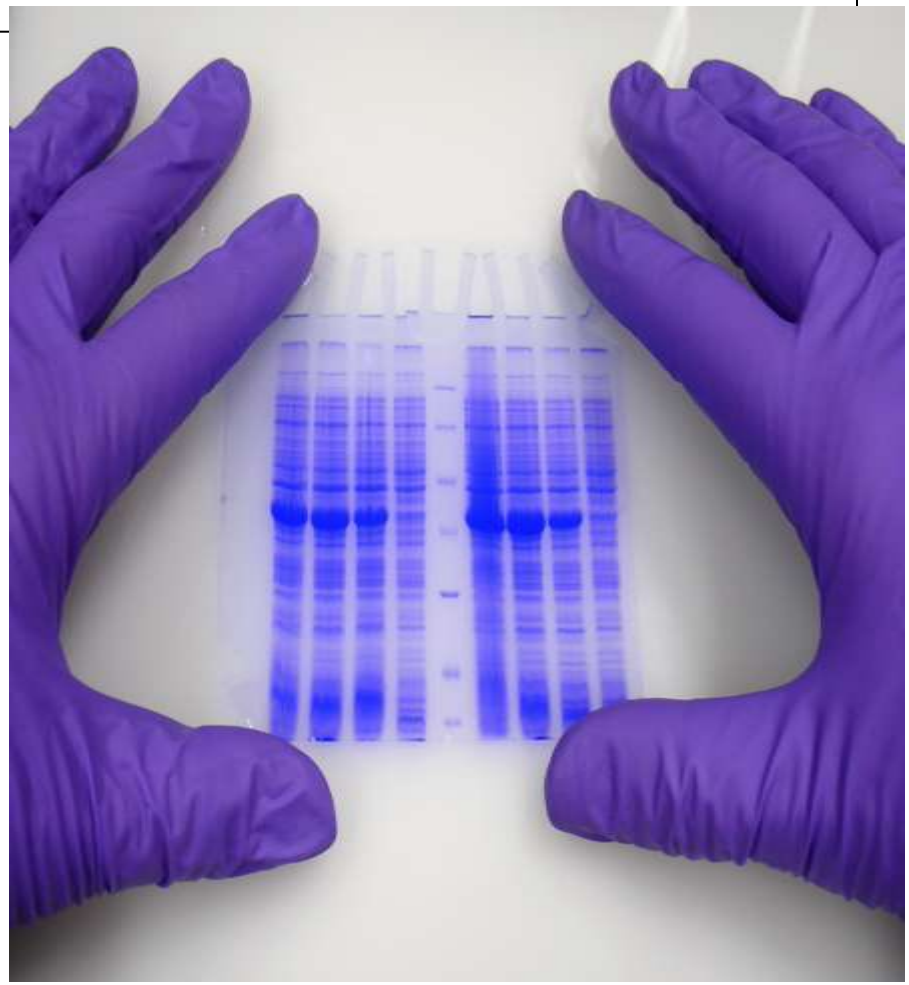
Objectives

- Brief overview of DNA and terminology
- Current technology
- Applications of molecular testing results



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DNA to Antigen

Nucleotides: A, G, T, C

DNA - template for duplication to mRNA

mRNA translated to form protein

Protein structure on red cell surface

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Molecular Basis of Blood Group Antigens

- Genes encoding 28 of 29 blood group systems
  - Only P1 remains to be solved
- 40 genes; over 1,100 alleles that encode the blood group antigens and phenotypes
- More than 300 antigens

Molecular changes that result in Antigen or Phenotype

- Single nucleotide change-most common
- Deletion or insertion of a nucleotide
- Deletion or duplication of an exon
- Deletion of a gene
- Gene crossover, conversion, recombinant event
- Absence of a required interacting protein
- Presence of a modifying gene
- Unknown

-Reid ME, Immunohematology 24: 2008, pp. 166-169.

Serology and DNA are complementary

- Serologically defined red cell types used to determine the molecular bases of variant forms of the gene.
- Molecular basis allows DNA analysis to be performed to predict the presence or absence of an antigen (Ag).
- Most antigens are result of SNP change.
  - PCR-based assays identify these changes

PCR: Polymerase Chain Reaction

- Used to amplify a specific region of DNA that contains the nucleotide that encodes the Ag
- PCR reaction mixture contains primers complementary to the gene of interest
  - Primer: 18 to 36 base pairs; designed to amplify short DNA segments
- DNA-PCR reactants in a thermal cycler
  - denature the DNA
  - combine with primers
  - stabilization. Repeat 25 to 35 times to amplify.

-Hue-Roye K, Vege S. *Immunohematology* 24; 2008, pp.170-175.

Significant Advances in Molecular Testing

- Gene targets have been validated
- High correlations with RBC phenotype
- Discrepancies between serologic and molecular results extensively investigated
- Manual testing replaced by automated platforms

Challenges in Molecular Blood Group Testing

- Numerous polymorphisms

  Ex.: ABO and Rh blood group systems

- Detection of common silencing mutations

  Examples:

  - *GATA* mutation that silences Fyb expression on RBCs
  - Two changes on the *GYPB* silence glycophorin B for Ss expression

Current Applications of DNA testing in the Blood Bank

- Typing multiply transfused patients
- Lack of rare or reliable antisera
  - Ex: Dombrock; Low frequency antigens-V, VS
- Reagent discrepancies
  - Contaminating specificities
  - Weak antigen expression
- Determining zygosity
- Testing fetal DNA
- High volume donor screening

Case 1

- Hospital performs ABO confirmation typing on RBC labeled Group O, Rh Positive

- Anti-A,B reacts 1+
- Donor history checked
  - 1st-time donor
  - Automated typing: Group O, Rh Positive

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Case 1: Serologic Investigation

	Antiserum				Reverse Cells			
	A	**B**	**A, B**		**A1**	**A2**	**B**	**O**
#1	**0/0**	**0/0**	**1+w/1+**		1+w/1+	0/0	1+/3+	0/0
#2	0/0	0/0	0/0					
#3	0/0	0/0	NT					
#4	0/0	NT	NT					

Readings: I.S./ 15' RT

#1-Forward typing reagents used by hospital

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Case 1: Investigation continues

Repeat forward testing with papain-treated donor cells:

All reagents remain negative except anti-A,B #1

Absorption-elution studies with anti-A and anti-B: No reactivity detected

Investigate donor mix-up: none found

Review of ABO testing: on previous donations by the donor

Case 1: Molecular results

- Two nucleotide deletions
  - position 261 characteristic of O alleles
  - position 467 characteristic of A2 alleles
- Genomic cloning and sequencing: weak A subgroup allele
  - ABO*AwO2 (350C,467T, and 1060 deleted C)

ABO genotype: *AwO2/01*

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Case 1 Molecular results *cont.*

- *AwO2* allele reported
  - Blood, 98: 1585-93, 2001.

- Expression of A antigen on these cells has only been detected as weak reactivity with reagents containing the ES15 monoclonal antibody .

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Case 1 Conclusion

- Donor is group A
  - Antigen not detectable by most reagents
  - Deferred from routine blood donation.
- What if the unit was transfused as group O?
  - No reports of transfusion reactions
- What if this person needs blood?
  - As a patient, classify him as group O

Case 2: Another ABO problem

- 32 y.o. woman with anemia and weakness
- Recently transfused in another state
  - History: group AB with anti-A1
- Strong anti-IH in plasma plus anti-Fya
- ABO results (Galileo): B Positive
  - A1 cells: 2+
  - B cells: 0

Case 2: Manual ABO testing

	Antiserum		Cells				
	Anti-A	Anti-B	A1	A2	B	Auto	O
I.S.	3+	4+	2+	NT	0	NT	NT
I.S.			2+	2+	0	0	2+
R.T.			2+	2+	0	0	2+
37C			1+	2+	0	0	0-1+

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Case 2: Continued

- ABO testing inconclusive
- Group O RBC units transfused over next few weeks
- Anti-IH remained strong
- Molecular testing: Predicted group A_2B
  - Genotype: *A2/B101*
- Group AB units could be crossmatched

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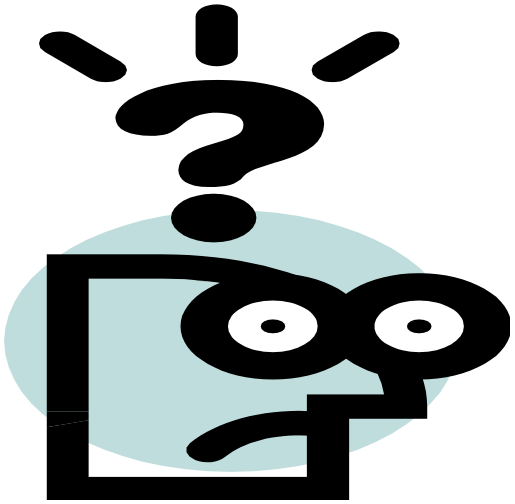
Case 3: Rh testing

- 62 y.o. man going to surgery
- Hospital results
  - ABO/Rh: A Positive
  - Antibody ID: Anti-D ??
  - DAT Positive



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Case 3: Serologic Investigation

- Anti-D typing: 3+; Rh control: 0
  - Other typings: Normal, no mixed field
- DAT: 3+ (Poly and IgG)
  - Eluate: Anti-D (4+)
- Plasma: Anti-D (2+)
  - Other specificities were excluded

Warm autoantibody with D specificity ??

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Case 3: Continued

- Patient RBCs treated with EDTA-glycine to dissociate the IgG  DAT Negative
- Patient plasma tested with his DAT neg. cells
  - **Weak** reaction-much weaker than other Rh Positive cells; control negative
- What does this mean?
  - Is pt. really Rh Positive?
  - Is he an Rh variant?

Case 3: Molecular results

- PCR results
  - Exons 4 and 7 present
  - Negative for the inactivating *RHD* pseudogene
  - Zygosity determination for hybrid Rhesus box
- Sequencing: Negative for weak D types
- Conclusion: *RHD* heterozygote
  - ***Not predicted to make allo anti-D***

Patient can receive Rh positive blood.

Anti-D was most likely autoantibody.

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Case 4: More D abnormalities

- 83 y.o. woman with low H/H
- History:
  - Transfused in past year
    - most recent 2 months ago
  - Reference Lab: O Pos, DAT-Wk +;
    - Warm autoantibody, Allo anti-E
- Current sample: O Positive
  - DAT: 2+ (Poly and IgG)

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Case 4: Serologic investigation

Cells	Plasma	Eluate
D+ E-	1+	3+
D+ E+	3+	3+
D- E-	0	1+

Case 4: Molecular Results

- *RHD* heterozygote
- *RHD* sequencing: nucleotide 520 G>A
  - Predicted to encode an amino acid change associated with **weak D type 33** (Reported in Taiwanese in 2003)
    - Persons not reported to be at risk for production of allo anti-D
    - NRLBGS previously tested another patient with this type who made allo anti-D

Patient should receive Rh negative blood

Case 5: Another blood group

- 77 y.o. man with myelodysplastic syndrome
- History provided by hospital :
  - Transfused about 4 weeks ago with 2 RBCs
  - A Pos; Negative antibody screen
- Current test results at hospital:
  - Antibody screen: 2 of 3 cells react 2+ at IAT
  - DAT: 1+ (poly and IgG)

Case 5: Serologic results

- DAT positive (recently transfused)
- Eluate: anti-Jka plus additional weak reactions
- Plasma: anti-Jka (PeG and Gel testing)
- Phenotyping: Cell separation performed
  - Kidd typings inconclusive
- Jk(a-) crossmatch compatible units sent

Case 5: Molecular results

- **Predicted phenotype: Jk(a+b+)**

  Review of serology:

  – **Consistent with autoantibody with Jka specificity**

  – **Jk(a-) blood is not required**

- Patient not seen again
- Patients with autoantibody specificities sometimes show panagglutinins when tested again

  – Specificity no longer honored

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Case 6: Back to Rh

- 57 y.o. woman with phlebitis
- History provided by hospital
  - –Transfused 8 months before
  - –O Pos with anti-C, -e and –K identified
- Two red cells ordered; Hgb. 8.4

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Case 6: Serologic Findings

- 1st time referred to Reference Lab
- O Pos; DAT negative
- Phenotype: C-, K-, **e weak,** Jk(b-), S-
- Plasma tested with cells negative for C, e + K
  - **Anti-Jkb and anti-S identified**
- **C-, e-, K-, Jk(b-), S- found in 9 of 10,000 donors--------Two units found and provided**

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Case 6-Additional Investigation

- Additional e typings:
  - 2 licensed sources
- Results indicate pt. may have weakened or variant form of e antigen
- Is the previously identified anti-e an allo- or auto-antibody?

Anti-e	Pt.	Pos. Ctrl.
#1	1+w	3+
#2	2+	4+

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Case 6: Molecular results

- Extensive analysis including sequencing the *RHCE* gene for exons 1 through 8.
- No changes associated with altered or partial e antigen expression and production of allo anti-e.
- If the anti-e shows characteristics of an alloantibody, Rh-cDNA testing can be done to rule out rare or new hybrid alleles.
  - Further serologic evaluation will be performed.

Case 7: Unexpected Benefit

- 31 y.o. pregnant woman
  - African-American
- Hospital results
  - Patient typed A Rh Positive
  - Found apparent anti-D and suspected partial D
  - No history of RhIg administration

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Case 7 Serologic Findings

- A, Rh Positive (D typing: 4+)
- DAT negative
- Rh phenotyping: C *inconclusive*, E-, c+, e+
- Plasma: Anti-D;
  - Apparent allo antibody
  - Does she have partial D?

Case 7: Molecular testing

- Partial ***RHD*DIVa-2****:* Encodes for low incidence Go^a antigen.
- SURPRISE……. Both *RHCE* genes are unusual
  - Altered e, VS+ V+, hr^B negative

Predicted phenotype*:*

Partial D+, C-E-c+ partial e+, VS+, V+, **hr^B-**

Transfuse Rh negative, compatible red cells

…..very conservatively!

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Case 8

- 48 y.o. woman with sickle cell anemia
- Hospital reported
  - 4 RBCs transfused 6 weeks ago
  - Anti-Fya previously identified
  - Suspect anti-C in eluate

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Case 8: Serologic Findings

- O Positive
- DAT: Weak Positive in Gel (Negative in tube)
- Hypotonic wash performed to obtain autologous red cells
  - C-, K-, Fy(a-b-), Jk(a-)
- Eluate: anti-C

Case 8: Additional Serology

- Plasma: 2+ reactions with all e+ cells
  - All e- cells are non-reactive; could not r/o C, K, Jsa
- Pt. types e positive (4+) and DAT is positive
  - Plasma reactions are stronger than DAT
- Provided e-C- units and recommended molecular testing to determine if anti-e is auto- or allo-antibody

More Case 8 Findings

- Plasma: Most e-, C-, Fy(a-) cells compatible
  - Everything ruled out except K and Jsa
- Why should she make anti-e?
  - Her red cells react 3+ with anti-e
- Perhaps her red cells have a variant form of e
- Cells lacking hr^B and hr^S are reactive
- Report “anti-e like”, anti-C, anti-Fya and unidentified reactivity

Case 8: Molecular Results

- *RHD: DIIIa/RHD (One partial and one normal D)*
- *RHCE:*
  - ***RHCE*ce^{s}:*** Associated with partial e, VS+, V-, hr^{B}- phenotype.
  - ***RHCE*cE***
- *Predicted phenotype:*

D+C- E+ c+ partial e+, VS+, V-, hr^{B}-

Good news!! Patient can receive

R2R2 (e-), Fy(a-) blood

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Donor Testing

- As donors are identified with unusual types, the information is added to the rare donor database (ARDP).
- Attempts made to make molecular matches for patients with anti- hr^{B} or anti-hr^{S}
  - hr^{B} negative blood is acceptable but a more exact match can be made using molecular results
  - Ex: *RHCE*ce(733G)* produces one hr^{B} – phenotype and *RHCE*ce(48C,733G) makes another*

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Study on prevention of alloimmunization in sickle cell patients

- Tournamille C et al. Transfusion 2010;50:13-19
- Studied sickle cell patients who typed C+
- Screened for alleles associated with partial C
- Patient history:
  - Alloantibodies produced
  - Determined the number of C+ transfusions

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Study *continued*

Group 1: Retrospective review

- Clinic patients n=1076
- 2/3 HbSS; others HbSC and S/βThal
- 22% typed C+
- 5% of C+ patients made anti-C

Study *continued*

Group 2

- 177 patients with sickle cell disease transfused in Paris area (Afro-Caribbeans0
- 49 had the (C)ce^{s} haplotype without C on the other haplotype-----had partial C antigen
- -37 of 49 received C+ blood

Of this group, 30% produced anti-C

Study *continued*

- Authors concluded
  - The need to detect partial C within C+ HbSS pts.
  - To prevent immunization in this group
- Study needs to be repeated in the U.S. HbSS patient population

Are our C+ HbSS patients at risk to produce anti-C?

Conclusions

- Serology will not be replaced by molecular testing in the near future

- Molecular testing is
  - a valuable addition to the tools available for solving serologic problems
  - the best way to match blood for patients with antibodies that are serologically difficult to define

References

- A Workshop on Molecular Methods in Immunohematology. Transfusion 2007; 47, July Supplement.

- Immunohematology 2008, Volume 24, No. 4

Happy St. Patrick’s Day

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