An Introduction to Blood Group Genotyping

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Disclaimer: All Blood Group Genotyping tests commercially available in the U.S. and Canada are for Research Use Only. Not for use in diagnostic procedures.
Agenda

• Source
• Techniques
  - Allele-Specific PCR
  - Allele-Specific Hybridization
  - DNA Sequencing
• Benefits
  - Typing of multiple antigens
  - Lack of commercial reagents
  - Inconclusive agglutination test
  - Unreliable agglutination test
  - Mixed-field agglutination
  - Serologic discrepancy
• Real Cases
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Source

WBC
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Techniques
Allele-Specific PCR


Techniques
Allele-Specific PCR


C-A-G-\text{>} \quad \text{<}-\text{C}-C-T

Techniques
Allele-Specific PCR

✓ C-A-G->
<-\textcolor{red}{C}-C-T

✗ C-A-G->
Techniques
Allele-Specific PCR


C-A-G-> <\underline{T}-C-T

Techniques
Allele-Specific PCR

X

√
< -\textcolor{green}{T}-C-T
Techniques
Allele-Specific Hybridization

C-A-G->
<-A-G-G

C-A-G->
<-A-G-G
Techniques
Allele-Specific Hybridization

C-A-G-\rightarrow
\leftarrow A-G-G

C-T-\underline{C}-T-C

C-A-G-\rightarrow
\leftarrow A-G-G
Techniques

Allele-Specific Hybridization

√ C-A-G->
C-T-\underline{C}-T-C <-A-G-G

X C-A-G->
<-A-G-G
Techniques
Allele-Specific Hybridization

C-A-G->
<-A-G-G

C-T-\underline{T}-T-C

C-A-G->
<-A-G-G
Techniques
Allele-Specific Hybridization

X

✓
    C-T-T-T-C <-A-G-G
Techniques

DNA Sequencing

C-A-G>
<-A-G-G
Techniques

DNA Sequencing

C-A-G>
<-A-G-G

little c
Techniques

DNA Sequencing

C-A-G>
<-A-G-G

Big C
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The Benefits of Genotyping are the Limitations of Serotyping
Benefits of Genotyping

Sickle Cell patient
Will need multiple transfusions throughout life
Likely to develop antibodies
Transfuse better matched units

Problem: Need to type for multiple antigens
Benefits of Genotyping

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Will need multiple transfusions throughout life
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Problem
Need to type for multiple antigens

Genotyping tests are multiplex:
A, B, D, C/c, E/e, Cw, Cx, V, VS, K/k, Kpa/Kpb, Jsa/Jsb, Jka/Jkb, M/N, S/s, U, Dia/Dib, Coa/Cob, Doa/Dob, Lua/Lub ...
Benefits of Genotyping

Transfused patient with antibodies
Serology work-ups do not explain reactivity
Choose to type for rare antigens

- Problem

Antisera not available or very costly
Benefits of Genotyping

- Transfused patient with antibodies
- Serology work-ups do not explain reactivity
- Choose to type for rare antigens

Problem: Antisera not available or very costly

Genotyping for rare antigens is identical (reagents, costs) to genotyping for common antigens
Benefits of Genotyping

Donor sample with borderline agglutination
Reproducible results
Due to low antigen expression

Problem

Need to label the unit as antigen positive or antigen negative
Benefits of Genotyping

Donor sample with borderline agglutination
Reproducible results
Due to low antigen expression

Need to label the unit as antigen positive or antigen negative

Genotyping distinctly detects variants that cause weak antigen expression: D, C/c, E/e, k, Fyx, U ...
Benefits of Genotyping

Patient with blood malignancy
Develops auto-antibodies
Agglutination is unreliable

Problem: Need to determine true type
Benefits of Genotyping

Patient with blood malignancy
Develops auto-antibodies
Agglutination is unreliable

Problem: Need to determine true type

Genotyping analyzes DNA, unaffected by the presence of antibodies on RBCs or serum
Benefits of Genotyping

Recently transfused patient
Bone marrow recipient
Mixed field agglutination

Problem

Agglutinating antigens from host or from graft?
Recently transfused patient
Bone marrow recipient
Mixed field agglutination

Problem

Agglutinating antigens from host or from graft?

Genotyping can analyze DNA from sources other than blood (e.g. cells in saliva)
Benefits of Genotyping

Patient typed as Rh D negative at institution X
Rh D positive at institution Y

Patient typed as Rh D negative with reagent X
Rh D positive with reagent Y

Problem: Need to resolve discrepancy
Patients typed as Rh D negative at institution X
Rh D positive at institution Y
Patient typed as Rh D negative with reagent X
Rh D positive with reagent Y

Need to resolve discrepancy

Benefits of Genotyping

Genotyping predicts antigens (e.g. weak D, partial D, Del) that yield variable results in agglutination tests
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Real Cases

• Case 1:

As a donor, JS was serotyped as A at the state blood center. As a state hospital patient later on, JS is serotyped as O. Genotyping is requested to resolve the discrepancy.
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As a donor, JS was serotyped as A at the state blood center. As a state hospital patient later on, JS is serotyped as O. Genotyping is requested to resolve the discrepancy.

• Genotyping shows that JS is O\textsubscript{2}/O\textsubscript{2}.

*It has been found and published that some O\textsubscript{2} individuals can express a certain amount of A antigen (homozygotes more than heterozygotes). The underlying basis is unknown.*

The phenotype predicted from the genotype is O or Weak A. Weak A expression explains the discrepant serology.
Real Cases

• Case 2:

A six year-old Thalassemia Major patient is serotyped as AB+. He receives 1 RBC unit every 3 weeks. Genotyping requested to confirm the serotype and predict his extensive phenotype.
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• Genotyping confirms the presence of A, B, RhD antigens and adds the following phenotypes:
  C+, c-, E-, e+, Cx-, Cw-, VS-
  K-, k+, Kpa-, Kpb+, Kpc-, Jsa-, Jsb+
  Jka-, Jkb+
  Fya+, Fyb-
  M+, N+, S-, s+, U+
  Dia-, Dib+, Doa-, Dob+, Coa+, Cob-, Lua-, Lub+
Real Cases

• Case 3:

A pre-natal African-American is found to have developed auto or allo-anti-D antibodies in serum. Genotyping is requested to determine whether she expresses a common or variant D type.
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- Genotyping of RHD detects polymorphism c.1048C in homo or hemizygosity. This polymorphism defines the variant allele RHD*DIVa-2, which encodes a Partial D phenotype. From it, administration of anti-D Ig is decided.
Real Cases

• Case 4:

A 47 year-old leukemia patient with no history of transfusions or bone marrow transplants shows a mixed field with several anti-A antibodies. A variant D phenotype is suspected. Genotyping is requested to confirm ABO and D types.
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A 47 year-old leukemia patient with no history of transfusions or bone marrow transplants shows a mixed field with several anti-A antibodies. A variant D phenotype is suspected. Genotyping is requested to confirm ABO and D types.

- Genotyping detects A₁ and O₁v alleles, from which an A phenotype is predicted. Genotyping also reveals the presence of the partial D variant RHD*DFR in homo or hemizygosity.
Real Cases

• Case 5:

A 63 year-old male with warm-auto hemolytic anemia, history of two bone marrow transplants (A & AB), and multiple recent transfusions (O+ & O- RBCs) shows positive antibody screen, positive DAT. Eluate reacts with all cells tested.
Real Cases

• Case 5:

A 63 year-old male with warm-auto hemolytic anemia, history of two bone marrow transplants (A & AB), and multiple recent transfusions (O+ & O- RBCs) shows positive antibody screen, positive DAT. Eluate reacts with all cells tested.

• Genotyping predicts the presence of A, B, D, c, E, e, k and 15 other minor antigens. Genotyping avoids antibody interference in antigen typing, but it cannot avoid interference from transplanted or transfused cells when performed on blood. That requires genotyping of cells from a different source (e.g. saliva).