THINKING OUTSIDE THE (GATA) BOX

GINA FOLK, MLS(ASCP) CM SBB CM

Community Blood Center
Save a Life. Right Here, Right Now.

New York Blood Center
OBJECTIVES

• Recognize the importance of identifying and differentiating anti-Fy^b_ from anti-Fy3 in the presence of the GATA box mutation

• List the enzymes used to aid in the identification of additional alloantibodies

• Discuss the benefits of genomic testing in antibody identification and provision of red blood cells
A SAMPLE ARRIVES...

- Sample arrived on 2-6
- 50 year old African American female
- Sickle cell disease with wounds on lower extremities
- Hgb 6.0
- 2 units ordered
- Hospital reports:
  - Group O Neg, negative DAT
  - Plasma reactive with all cells in tube tests with LISS, PEG and solid phase
  - Transfused 2 units 1 week ago
  - Patient just moved from California
    - No history in this area

△New York Blood Center
INITIAL CBC TESTING

- **ABORh:** O Neg
- **DAT:** Negative

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- **Antibody screen:**
  - 5” RT testing looked like anti-M
  - All cells tested in PEG IAT positive 2-4+
  - Autocontrol negative
# M-NEGATIVE PANEL

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What we can use to help identify any and all the antibodies?

- Phenotype
  - Know what the patient is antigen negative for
- DTT
  - Destroys Kell, Dombrock, Lutheran antigens
- Ficin
  - Destroys Duffy and MNSs antigens
- EGA
  - Destroys HLA and Kell antigens
# FICIN PANEL

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**PHENOTYPE**

- **Phenotype:**

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- Patient can make antibodies to E, K, Fy\(^a\), Jk\(^b\), S, and M antigens

- Antibody to Fy\(^b\) antigen not as likely as African American and most likely has GATA box mutation
  - Will need genotype testing performed to verify
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ANTIBODIES IDENTIFIED

- Selected cell panels PEG IAT and ficin treated cells show:
  - Anti-E 2+ in PEG IAT
  - Anti-K 1+ PEG IAT and 3+ ficin IAT
  - Anti-Fy\textsuperscript{a} 2+ PEG IAT
  - Anti-Jk\textsubscript{b} 3+ PEG IAT and 3-4+ ficin IAT
  - Anti-S 3+ PEG IAT
  - Anti-M 4+ RT and 4+ PEG IAT
    - Did not prewarm clinically significant
  - 4 extra reactions micro to 1+
    - 1 ficin treated cell IAT and 3 cells PEG IAT
    - 2 of 3 cells in PEG were negative after EGA treatment
      - Most likely due to HLA antibody
TRANSFUSION

- Sent 2 units (1 liquid and 1 deglyced)
  - Group O negative
  - E-, K-, Fy(a-), Jk(b-), S-, M- and nonreactive with patient’s plasma

- Also sent sample to NYBC for HEA testing
  - Verify phenotype
  - Obtain extended genotypes on antigens we don’t have antisera to type
  - Verify if patient has GATA box mutation
**Human Erythrocyte Antigen (HEA) Phenotype by DNA Analysis Report**

**Sample ID:** [Redacted]

**Chip ID:** HEAC1763_6

**Lot #:** 16-122

**Chip Read Date:** 11 Feb 2016 11:16

**Print Date:** 11 Feb 2016 12:09 EST

**Software:** V4.1.4

**Generated by:** NYBC Admin

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**LEGEND**

- (+)*: Possible hybrid allele. Additional serological testing recommended for Ig C.
- (0)*: GATA silencing mutation present
- CV (Coefficient of Variation): CV of intensities above recommended maximum
- HS (High Background): Signal intensity above recommended maximum
- LC (Low Intensity): Algorithm unable to confidently predict result
- LS (Low Signal): Signal intensity below recommended minimum
- NTD (No Typing Determined): Typing was not able to be determined
- Var: U variant detected
- W: Weak expression

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Kim Hue-Roye
Senior Molecular Biologist

Simitha Vege, MS
Manager, Genomics

This Precise Type HEA (human erythrocyte antigen) test has been licensed by the Food and Drug Administration (FDA) to predict the blood group antigen profile in a patient or donor. There are situations where testing of DNA may not reflect the red cell phenotype. Specifically, nucleotide changes that inactivate gene expression or rare variant alleles may not be detected. Results obtained from DNA isolated from leukocytes or other hematopoietic cells may differ from DNA isolated from other tissues in persons with a history of transplantation.
HEA RESULTS

• Confirmed serologic typing

• GATA mutation present
  • Patient not expected to make anti-Fy\textsuperscript{b}

• C(+)\textsuperscript{*} Result on C typing
  • Indicates sample may have a hybrid D-CE-D encoding partial C or may have partial D antigen
  • A C+ typing would indicate the presence of an r’S i.e. (C)ce\textsuperscript{S} haplotype associated with partial C
GATA BOX MUTATION

• Substitution at the GATA of FY*B promoter (-67 T→C) reported in Fy(a-b-) black individuals
  • Mutation disrupts binding site for GATA-1 erythroid transcription factor
  • Results in silent FY*B allele in erythroid cells only

• GATA mutation considered responsible for most cases of Fy(a-b-) in black populations
  • Also occurs in other populations including some non-Ashkenazi Jews, Arabs, Brazilians, Romanies

• Fy(a-b-) is rare in Caucasian populations
  • Occurs due to point mutations that encode premature stop codon in FY*A or FY*B
WE MADE IT!
A SAMPLE ARRIVES…

- Sample arrived on 2-11
- Hgb 5.0
- 2 units ordered
- Hospital reports:
  - Patient now has a weakly positive DAT with IgG and C’
- Inquired about signs of transfusion reaction
  - Per hospital only saw plasma—slightly icteric
  - No haptoglobin was tested
  - Bilirubin: normal range 0.3-1.2 in adults
    - 2/5 2.0
    - 2/8 2.9
    - 2/9 2.4
INQUIRED ABOUT PAST HISTORY

- Patient had history of moving from California
  - Patient was seen 5 years ago in Freemont, CA

- Patient has been at current facility since 1-28
  - On 1-28 the antibody screen was positive but couldn’t identify anything
  - Solid phase was reactive with all cells but negative in tube tests
  - Transfused 2 random group O Rh negative units
    - 1 unit had no antigen typing history
    - 1 unit was historically C-, E-, K-, Fy(a-), Jk(b-), s+
AMERICAN RED CROSS (ARC)

- History from ARC Northern CA region
  - Sample date 2-13-10
    - DAT Positive: C’ (microscopic)
    - Eluate not indicated
    - Probable Rh genotype \( r'^S \) variant by molecular testing
    - Phenotype was the same as ours
    - Antibodies identified
      - Anti-Fy\(^a\)
      - Anti-Jk\(^b\)
      - Probable anti-Do\(^a\)
      - HLA
      - Anti-M (clinically significant)
Sample sent to the ARC Reference Lab in Philadelphia 6-6-10

- **DAT Positive:** Poly (m+), IgG (m+), C3 negative
- **Eluate:**
  - Couldn’t definitively identify any antibodies, and could not rule out all alloantibodies. Sample was QNS for further testing
- **Plasma**
  - Anti-M
  - Anti-Do<sup>a</sup>
  - Anti-E
  - Anti-Js<sup>a</sup>
  - ID at facility before sending to reference laboratory
    - Not demonstrating in current sample
      - Anti-Fy<sup>b</sup>
      - Anti-S
      - Anti-K
    - Not re-identified
      - Anti-Fy<sup>a</sup>
      - Anti-Jk<sup>b</sup>

- **CBC Identified**
  - Anti-E
  - Anti-K
  - Anti-Fy<sup>a</sup>
  - Anti-Jk<sup>b</sup>
  - Anti-S
  - Anti-M
  - HLA
WHAT A MESS!

- Patient had 2 transfusion reactions
  - 1st transfusion reaction
    - Random O Neg units given by hospital on 1/28
  - 2nd transfusion reaction
    - E-, K-, Fy(a-), Jk(b-) S-, M- units given on 2/6
    - Do\(^a\) typings of these units were unknown

- Both could have been avoided if patient’s history was known
DAT: Positive IgG and C’
  - Microscopic and mixed field reactivity
Acid Eluate:
  - Anti-M
  - Anti-Fy\textsuperscript{b}
  - 2 cells extra reactivity (Do\textsuperscript{a} typings unknown)
Plasma:
  - Anti-Fy\textsuperscript{b}
  - Anti-Do\textsuperscript{a}
  - HLA
  - 1 cell extra reactivity (Do\textsuperscript{a} typing unknown)
ANTI-FY$^b$ AND ANTI-FY$^3$

- **Anti-Fy$^b$**
  - React with only Fy(b+) cells
  - Antigens **destroyed** by ficin

- **Anti-Fy$^3$**
  - Reacts with any cell that is Fy(a+), Fy(b+), and Fy(a+b+)
  - Antigen **not destroyed** by ficin

- **Observed reactivity at CBC**
  - 2-6 sample
    - anti-Fy$^a$ in plasma
    - No reactivity with Fy(b+) cells
  - 2-11 sample
    - Anti-Fy$^b$ in eluate and plasma
    - Anti-Fy$^a$ not demonstrating
    - No reactivity observed with Fy(a+) cells
    - Reactivity removed when testing with ficin treated cells
DUFFY SEQUENCING

- NYBC report:
  - Promoter and exon 1: -67t/c
    - homozygous for GATA mutation associated with Fy(b-)
  - Exon 2: 125A/A (42Asp) FY*B/B
  - FY genotype: Fy*02N.01/*02N.01
    - Predicted phenotype: Fy(a-b-)
  - Comments:
    - FY sequencing confirmed the patient is Fy(a-b-). No additional changes were found and patient would not be predicted to make alloanti-Fy\(^b\). Like anti-Fy3, we have not found a biological explanation for these apparent FY specificities.
EASY AS 1, 2, 3...RIGHT?

- Antibodies identified:
  - Anti-Fy\(^b\)
  - Anti-M
  - Anti-E
  - Anti-K
  - Anti-Fy\(^a\)
  - Anti-Jk\(^b\)
  - Anti-S
  - Anti-Do\(^a\)
  - Anti-Js\(^a\)
  - HLA
  - Other: extra unknown reactivity

- Clinical Significance:
  - Anti-Fy\(^b\)=? Not sure if allo or auto anti-Fy\(^b\).
    - Honor Fy\(^b\) antibody and give Fy\(^b\) negative as we are unsure
  - HLA antibodies are not associated with accelerated red cell destruction
  - Other: unknown additional reactivity. The clinical significance is unknown.
  - All other antibodies are clinically significant

\(\text{New York Blood Center}\)
FINDING BLOOD

• 2 units had been ordered
  • O Neg, E-, K, Fy(a-b-), Jk(b-), M-, S-, Do(a-), Js(a-)
  • Still may not be compatible due to the extra reactivity and HLA antibody
• No units available at CBC liquid or frozen
• Options:
  • Called NYBC
  • Requested units through American Rare Donor Program (ARDP)
• Another sample arrived almost a year later in January
  • No new alloantibodies identified
  • Still no units available
UNANSWERED QUESTIONS

• How close do we need to monitor the Partial C?

• Has GATA mutation so shouldn’t make anti-Fy<sup>b</sup>

• Was it really anti-Fy<sup>b</sup> specificity or anti-Fy<sub>3</sub> or something else?
  • Negative ficin treated cells with plasma
  • Negative Fy(a+b-) cells in eluate.

• Does the patient have some unknown mutation or altered Fy<sup>b</sup>