Two is better than one, when detecting weak/partial D

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Background

- The accurate identification of D variants in patients is crucial for ensuring appropriate transfusion and immuneprophylaxis strategies. The AABB/CAP working group recommended in recent years to use two Anti-D reagents to assist in accurately determining the Rh status of patients.
- By analyzing the performance of different anti-D reagents/methods compared to molecular testing results, an understanding of their efficacy in detecting D variants and their potential implications for clinical practice can be obtained. This retrospective study was to optimize method selection, test algorithms, and improve diagnostic accuracy in transfusion medicine.

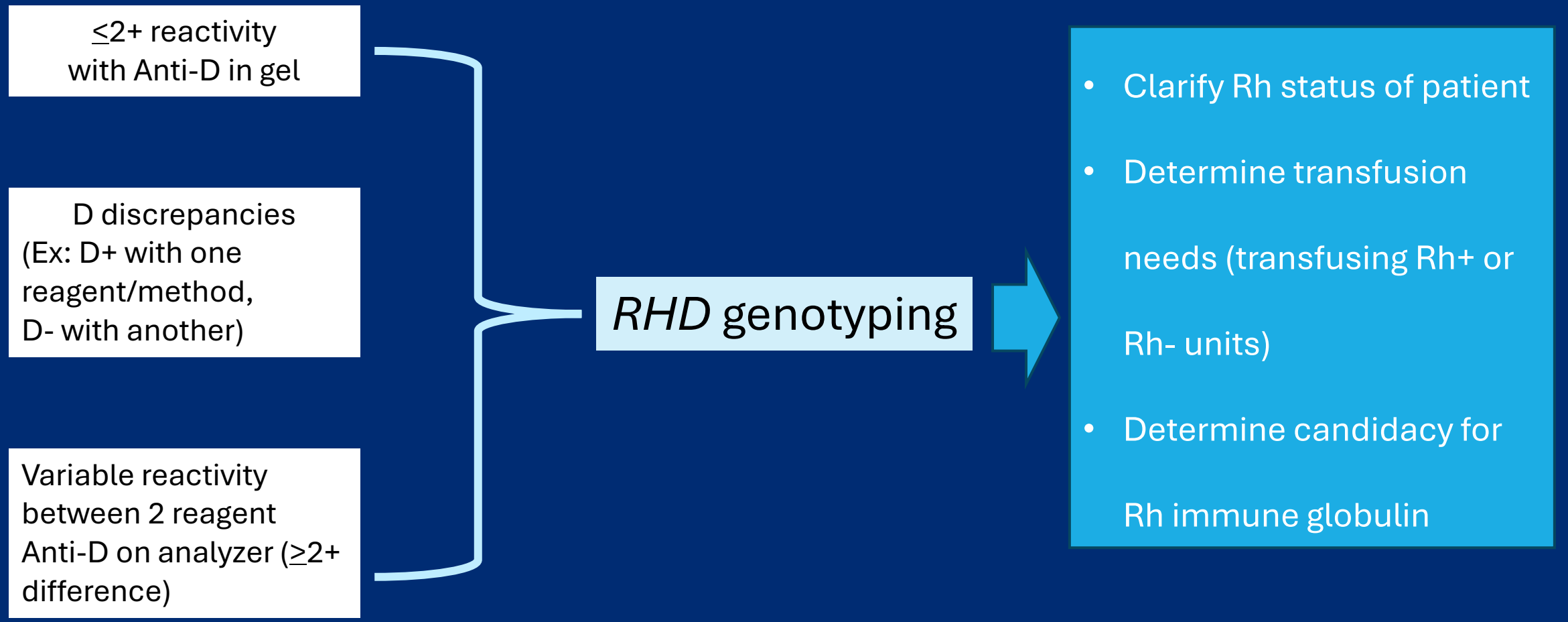
Background

- Since 2020 our hospital has been sending out weak reacting D patients for molecular testing to better treat these patients.
- We previously used gel technology, both manual and Provue analyzers QuidelOrtho (San Diego, CA). Our policy is to send any samples that anti-D reacts ≤2+ in gel out for genotyping.
- In 2022 our facility switched our automated analyzers. We use these to determine patients' ABO/Rh type, Echo Lumena (Werfen, Norcross, GA). This analyzer currently employs 2 different anti-D clones (D4 & D5) using hemagglutination.

Study Design

- Any questionable/discrepant results were sent for molecular testing at (Community Blood Center, Kansas City, KC) to provide a reference method and clarify the Rh status of the individual. This study compares molecular, gel and Hemagglutination methods to analyze trends.

Samples Sent for RHD Genotyping



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Interpreting the *RHD* genotyping results

RHD Genotype Results	Patient entered as	Transfusion Recommendation	RhIG candidate?
Weak D types 1, 2, 3, 4.1	Rh+	Rh+	No
Weak D type 4.0	Rh+	Rh+ (Rh- if currently pregnant)	* Determined by clinician
All other weak D or partial D	Rh-	Rh-	Yes
Conventional *RHD*	Rh+	Rh+	No

- Sandler et al. Transfusion. 2015;55(3):680-689.
- Flegel et al. Transfusion. 2020;60(4):855-859.
- Westhoff et al. Blood Transfus. 2019;17(2):91-93.

Results

- During approximately a 3-year study, 76 samples were sent for molecular testing.
- Of those tested with gel vs Hemagglutination, 32 of the 33 gave 1+ to 3+ weaker reactions with gel technology.
- Also, 18% of these samples were identified as variants and were classified as weak partial D 4.0, capable of producing anti-D.
- A weak partial D type 4 refers to a specific subtype of the partial D antigen, a variant of the of the RhD blood group antigen that has a reduced expression on red blood cells and carries a risk of causing alloimmunization.

Results

D Clone-Gel	D4 Clone-Hemagg	D5 Clone-Hemagg	Molecular	Race/Ethnicity	D Clone-Gel	D4 Clone-Hemagg	D5 Clone-Hemagg	Molecular	Race/Ethnicity
2	0	0	Weak D Type 2	Caucasian		2	2	Weak Partial 4.0	Other
2	0	0	Weak D Type 1	Caucasian	3	0	0	Partial D	Caucasian
3	2	1	Partial D	*African American		0	0	Weak D Type 1	Caucasian
2	0	0	Weak D Type 2	Caucasian		0	0	Weak D Type 2	Caucasian
3	0	0	Weak D Type 2	Caucasian		1	2	RHD Positive	African American
	2	1	Weak D Type 1	Caucasian	4	2	2	Weak D Type 1	African American
	2	1	Partial D	African American		0	0	Weak D Type 1	Caucasian
3	1	0	Weak D Type 1	Caucasian		1	0	Partial D	African American
3	1	1	Weak D Type 3	Caucasian	2	0	0	Weak D Type 2	Caucasian
3	2	1	Weak Partial 4.0	African American	3	2	2	RHD Positive	Hispanic/Latino
3	1	1	Weak D Type 3	Caucasian		2	2	RHD Positive	Caucasian
3	1	0	Weak Partial 4.0	African American		1	1	Weak D Type 3	Caucasian
	1	2	Weak Partial 4.0	African American	3	1	0	Weak D Type 1	Caucasian
	1	0	Partial D	*Caucasian		2	1	Partial D	African American
3	2	2	RHD Positive	Caucasian		1	0	Partial D	Caucasian
	1	0	Weak D Type 1	Caucasian		2	2	RHD Positive	Caucasian
	2	2	RHD Positive	Mixed Race		1	1	Weak D Type 1	Caucasian
	2	2	Weak Partial 4.0	Caucasian	3	1	0	Partial D	African American
3	1	2	Partial D	Caucasian	4	1	1	Weak Partial 4.0	*African American
	1	1	Weak Partial 4.0	African American		1	0	Weak D Type 1	Caucasian
	3	1	RHD Positive	Caucasian		0	0	Weak Partial 4.0	African American
3	3	1	Weak Partial 4.0	*African American	4	1	0	Weak Partial 4.0	Multiracial
3	2	2	RHD Positive	Caucasian		2	1	Partial D	African American
	1	0	Partial D	*African American		2	2	RHD Positive	African American
	1	0	Weak D Type 1	Caucasian	3	2	1	RHD Positive	African American
3	1	0	Weak D Type 3	Caucasian		2	1	Partial D	Caucasian
	3	1	Partial D	African American	3	2	2	Partial D	Caucasian
3	1	0	Weak Partial 4.0	Caucasian	3	2	1	Weak Partial 4.0	African American
	0	0	Weak Partial 4.0	African American	3	1	0	Weak D Type 1	Caucasian
	2	1	Weak D Type 33	African American	3	1	0	Weak D Type 1	Caucasian
	0	0	Weak D Type 2	Caucasian		1	1	Weak Partial 4.0	Unknown
4	0	0	Weak D Type 2	Hispanic/Latino		1	0	Partial D	African American
4	3	1	Weak Partial 4.0	African American		1	1	Partial D	African American
3	2	1	Partial D	African American		1	0	Weak D Type 1	Caucasian
3	1	1	Partial D	African American		1	1	Partial D	Caucasian
	1	1	Weak D Type 1	Caucasian		2	2	RHD Positive	African American
	2	2	RHD Positive	African American		1	0	Partial D	Caucasian
	0	0	Weak D Type 1	*Caucasian		1	0	Weak Partial 4.0	Caucasian

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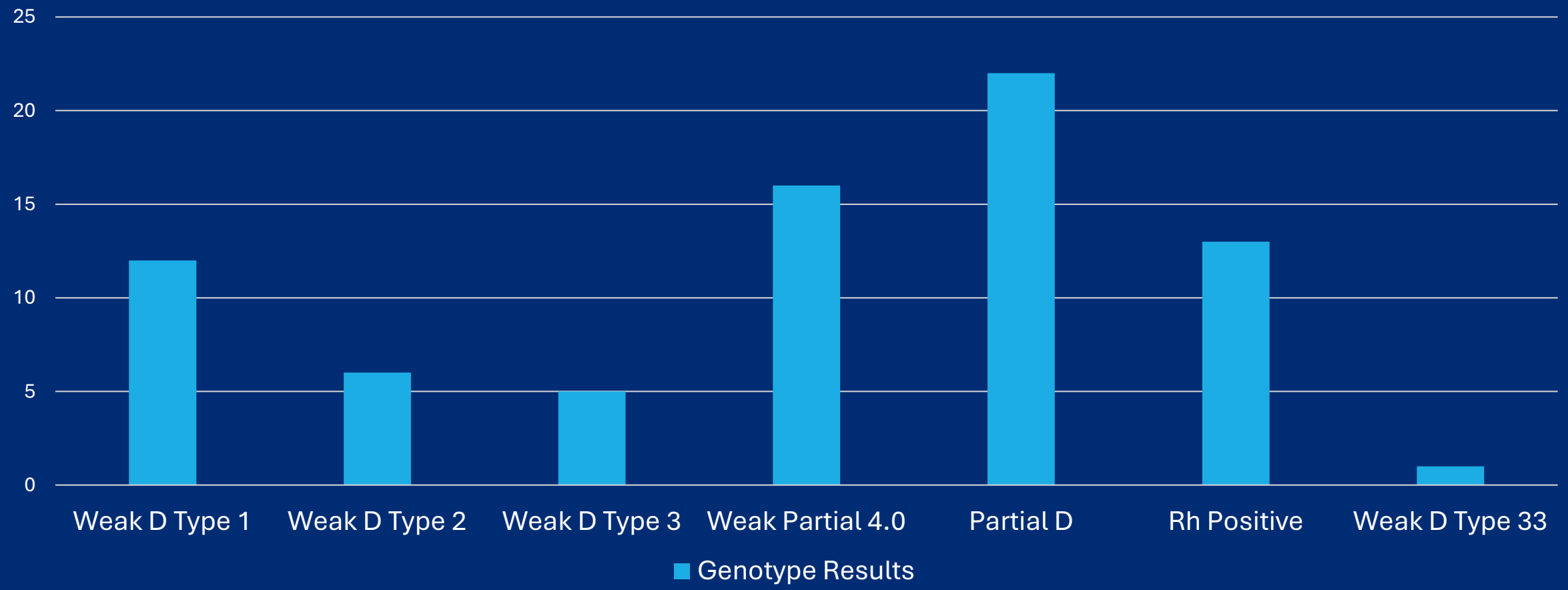
Results



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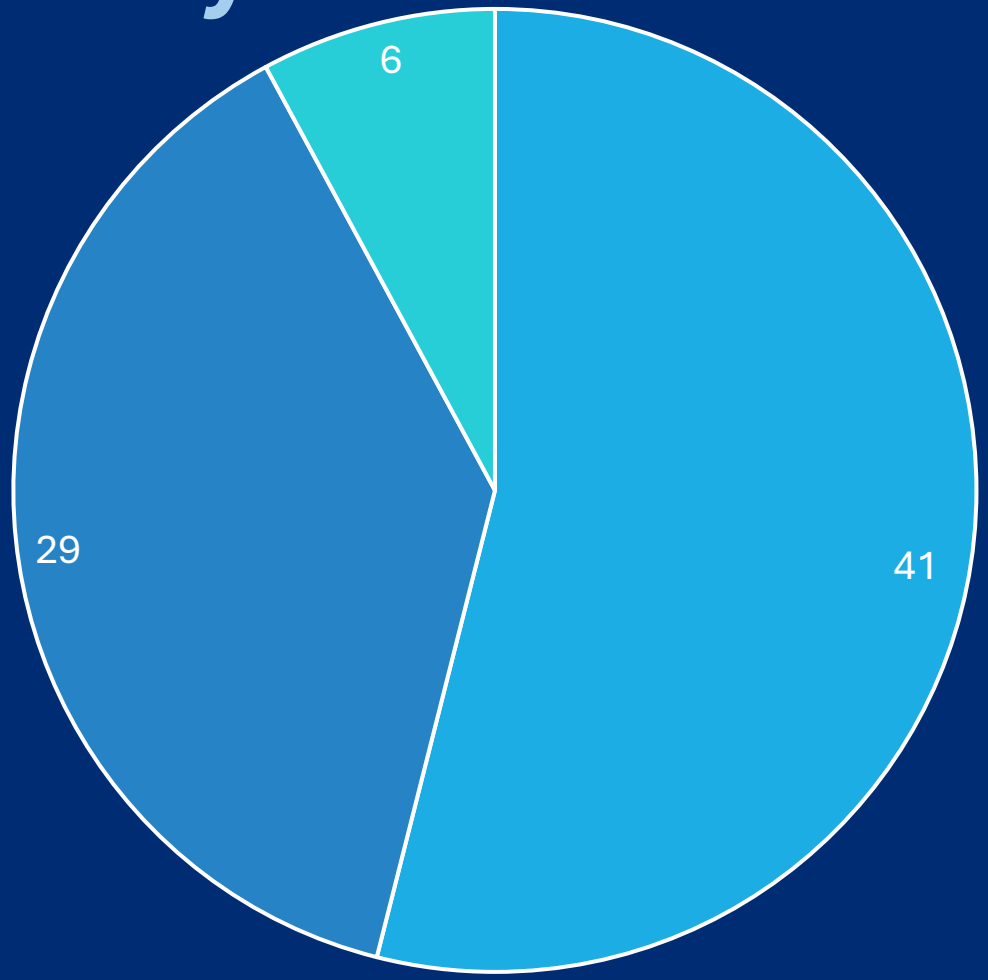
Results by Ethnicity



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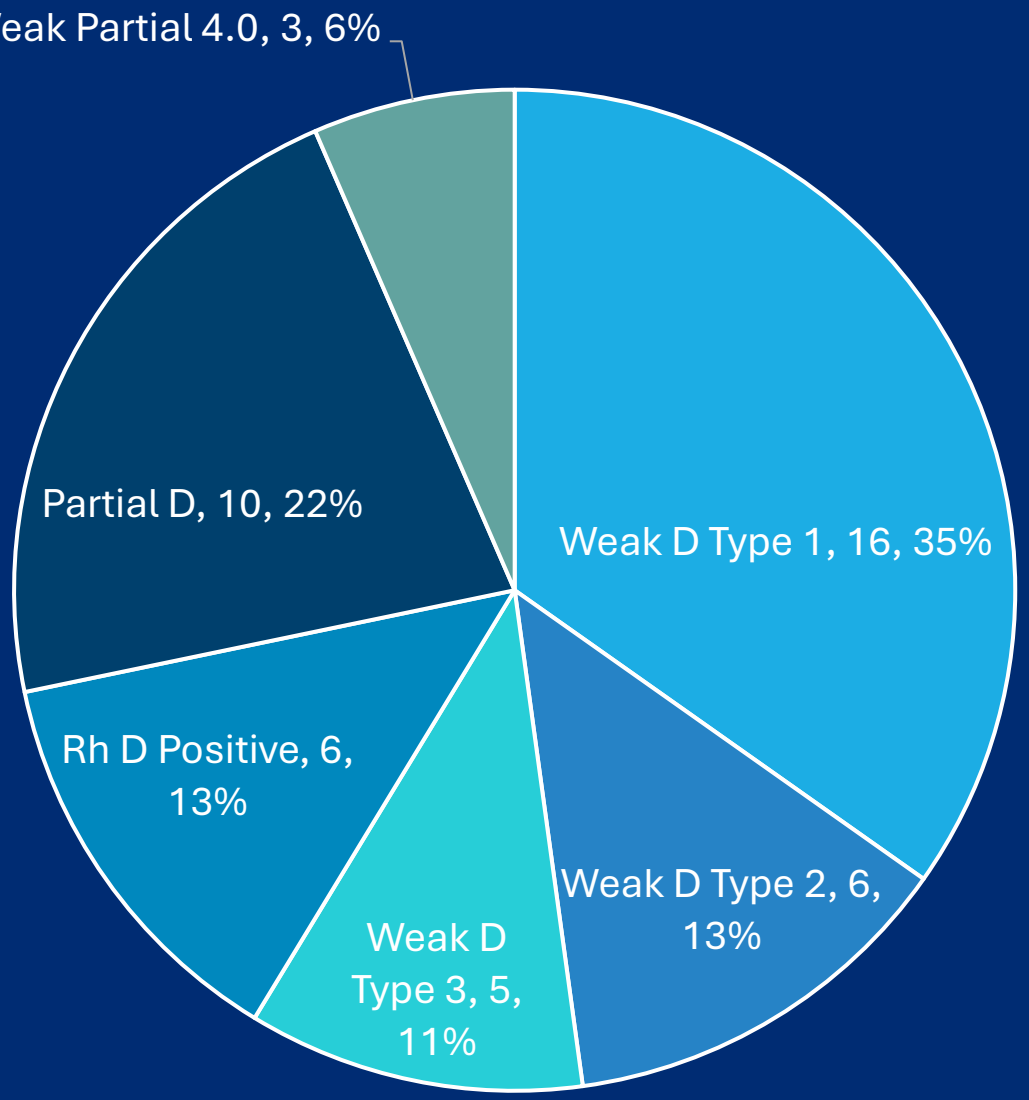
Caucasian



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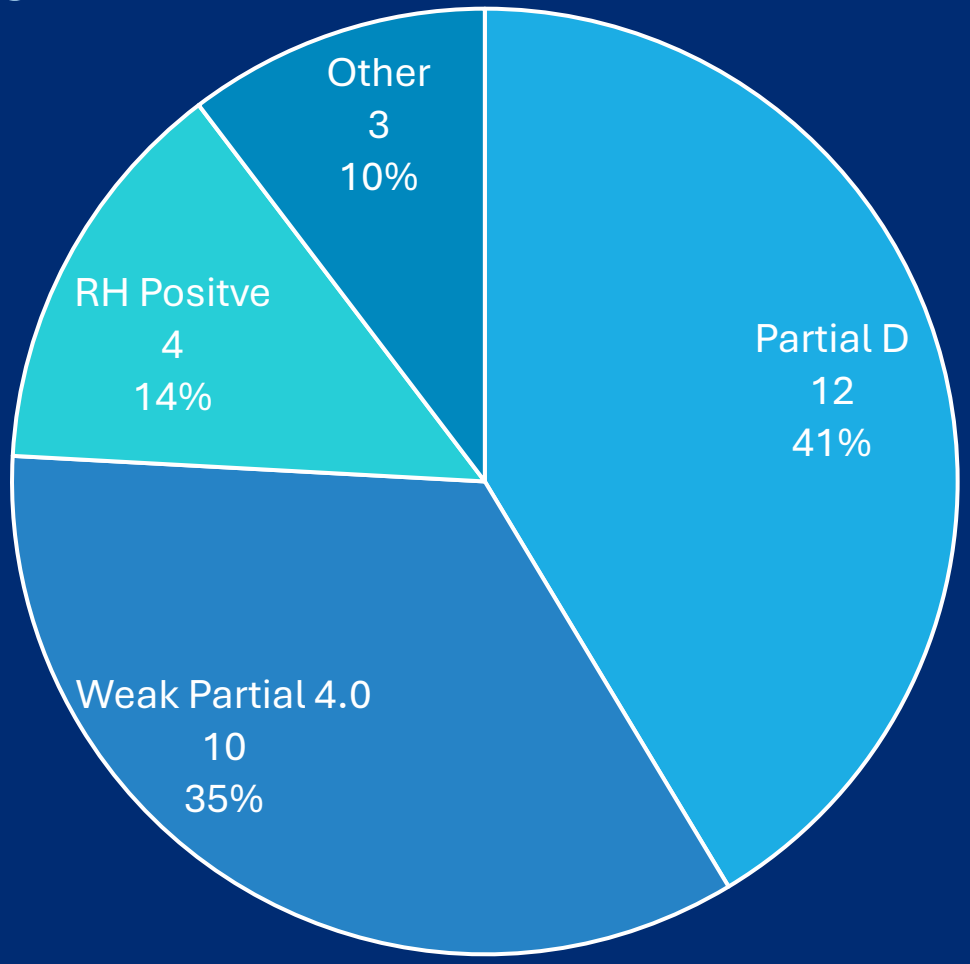
African American



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Type Conversion

- Of the 18 samples that were previously typed in Gel (2+ - 4+ positive)
  - 5 were Weak D Type 1
  - 4 were Weak D Type 2
  - 3 were Weak D Type 3
  - 3 were Partial D
  - 2 were Rh Positive
  - 1 was Weak Partial 4.0

- Resulting in changing 4 patient's blood types (weak partial 4.0 patient was pregnant)

RhIG utilization

- Based on the genomics results:
- 15 patients that would have received RHIG did not require this injection.
- 30 patients that would not have been considered Rh D positive were given RHIG.
  - Of the 14 patients identified as Weak Partial 4.0, 8 of them were currently pregnant and were given RHIG out of an abundance of caution.
  - 21 of these were Partial D and would have been categorized as Rh positive without this testing.

Conclusions

- This retrospective study affirms the AABB/CAP Working group's recommendation of utilizing two anti-D reagents to assist in the accurate detection of D variants.
- Our findings illustrate that our gel testing reagents tended to overcall D variants as Rh positive. This could potentially lead to the omission of Rh prophylaxis and the mistransfusion of Rh-positive products (39% of the samples sent would have been misclassified with traditional serological typing).

Conclusions

- Notably, 18% of the gel tested samples were misidentified and were actually variants that were weak partial D 4.0 types capable of producing anti-D.
- Formation of anti-D antibodies can compromise future transfusion safety and pregnancy outcomes. The study underscores the necessity for careful reagent selection and incorporating molecular testing to improve transfusion practices for this population of patients.

Conclusions

- Our lab has found the use of two anti-D clones has likely increased the detection weak D and other variants.
- Utilizing a polyclonal anti-D reagent like those found in many gel cards will likely mask weak or variant activity.
- We will continue to send these patients out even after our study since we believe this to be the best care for our patients.

Questions?

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