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# Updated Evaluation of RhD Status Among Women of Child-Bearing Age in Detroit, Michigan

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## ABSTRACT

***Objectives:*** *The Rh blood group system is one of the most important and immunogenic blood group systems after the ABO blood group system and, like other blood group antigens, it follows ethnic and racial trends. However, when it comes to D variants—partial D and weak D— most of the cohorts studied in the literature have been of European descent. This study aimed to discover the variant D trends in Detroit, Michigan, with an emphasis on Black communities.*

***Methods:*** *From 2016 to 2018, there were 102 patients (women of childbearing potential: < 50 years) at Henry Ford Hospital that had serologic D discrepant testing. These patients were sent out for molecular* RHD *determination.*

***Results:*** *In total, 12.7% of patients were characterized as RhD positive and 87.3% of patients were characterized as RhD variants (nominated as RhD negative at our institution).*

***Conclusions:*** *Our predominantly Black cohort sheds light on the diversity of the RhD antigen. The majority of Blacks were classified as RhD variants (RhD negative nomination at our institution). Therefore, molecular testing for this patient population with serologic RhD discrepancies is paramount to properly manage their obstetric care.*

**Key Points**

- Knowing that Detroit's racial and ethnic composition does not fit the European cohorts that are prevalent in the literature, we decided to quantify the prevalence of variant RhDs in this population.
- *RHD* alleles vary among various racial and ethnic groups.
- Women of childbearing potential in Black communities are at an increased risk for having serologic D antigen discrepancies that are consistent with potential alloimmunization and HDFN.

The Rh blood group system is one of the most important blood group systems after the ABO blood group system. Its implications are interdisciplinary and reach from transfusion medicine all the way to fetal-maternal medicine. As is known, Rh alloimmunization is a significant cause of morbidity during pregnancy, as it causes hemolytic disease of the fetus and newborn (HDFN).[1] Typically, individuals are either positive or negative for the D antigen. Alloimmunization can occur when the individual is RhD negative, hence pregnant women are given prophylaxis with Rh immune globulin (RhIG) to prevent alloimmunization and HDFN.[2] Problems arise, however, when individuals—especially pregnant women—are classified as having a D variant.[1]

The D antigen class includes 3 broad categories: RhD positive, RhD negative, and RhD variant. The RhD variant includes weak D or partial D. In standard blood bank practice, serologic testing—via automation or tube testing—can help detect potential RhD discrepancies in patients. Testing using 2 different clones of monoclonal anti-D reagent provides the ability to detect discrepancies in RhD patient samples. Discrepancies take on the form of an undetermined strength (usually characterized by a question mark) in one or both of the monoclonal anti-D

reagents. Patients with these results at our institution are classified as having a Rh typing problem. However, only molecular testing can differentiate the *RHD* gene as being fully expressed or having a variant expression.[3]

Most studies exploring weak D phenotypes and genotypes are of European cohorts; therefore, many published studies do not broadly address Black populations.[4,5] However, it has been shown that *RHD* allele frequencies vary among various racial and ethnic groups.[6-10] It is crucial to point out that Black populations have a higher prevalence of particular *RHD* alleles; therefore, these patient populations do not follow the standard literature—and clinical recommendations that have been crafted from the literature—so it is imperative that population-specific policies regarding genotypes, molecular results, blood products given, and RhIG are followed.[11]

RhD discrepancies pose the most threat to women of childbearing potential (designated as women <50 years at our institution). Depending on the *RHD* variant identified through molecular testing, these women have the potential to develop anti-D if exposed to the RhD antigen during pregnancy.[12] In these patients, it is vital to assign the proper D-antigen status to determine if they can receive RhD-positive or RhD-negative RBCs safely or, if pregnant, to determine if RhIG prophylaxis is necessary.[3] Detroit has a unique and diverse patient population, and Henry Ford Hospital has developed a pilot program to identify the genetic *RHD* status of qualifying RhD-discrepant patients. Determining the *RHD* status of this cohort of women will not only aid in accurately conserving RhD-negative RBCs but also allow for the proper administration of RhIG prophylaxis in this specific patient population, when applicable. In addition, we aimed to report the prevalence of both partial and weak Ds in the patient population served at our medical center located in Detroit. For practical purposes at our institution, we nominate women at risk of making an allo-anti-D as RhD negative and women who do not have the possibility of making an allo-anti-D as RhD positive.

## Materials and Methods

### Serologic Analysis

Beginning January 2016, patient samples showing inconclusive results with anti-D through automated testing were subject to manual serologic investigation via tube testing. Automated analysis was conducted on an Immucor NEO. Direct hemagglutination

microstrips were used for blood typing: strips included a monoclonal control, anti-A, anti-B, anti-D-series 4, anti-D-series 5, A1 cell, and B cell well. All reagents used were from Immucor and all reagent information was provided on package inserts. Anti-D-series 4 was a blend of monoclonal immunoglobulin M (IgM) and IgG anti-D from human/murine heterohybridoma (MS201 and MS26). Anti-D-series 5 was a blend of monoclonal IgM and IgG anti-D from human/murine heterohybridoma (Th28 and MS26). Anti-A and anti-B were murine monoclonal reagents. A1 and B cells were a 2% to 4% suspension of pooled C-D-E- RBCs. Agglutination of the patient RBCs with anti-A, anti-B, anti-D-series 4, or anti-D-series 5 indicated the presence of the corresponding antigen, and agglutination of patient serum with A1 or B cells established a positive test. Patients who had an anti-D-series 4 and anti-D-series 5 numerical value of greater than or equal to 65 were interpreted as Rh positive. Patients who did not meet this qualification moved onto manual testing.

Patients who qualified for manual analysis had their RBCs tested with the anti-D monoclonal reagent validated for tube testing. This process is detailed in ❚Figure 1❚.

### Samples

This study was approved by the Institutional Review Board at Henry Ford Hospital. At Henry Ford Hospital, there were 39,048 type and antibody screen samples in 2016, 41,534 in 2017, and 42,537 in 2018. Since our study sample ended on June 20, 2018, there were approximately 21,269 samples in 2018. In total, during this study period, there were approximately 101,851 samples. In total, there were approximately 101,851 samples. These numbers include repeat patients. A total of 106 patients showed inconclusive automated anti-D results from January 1, 2016, to June 20, 2018. Only women of childbearing potential (classified as <50 years at our institution) were included in the final analyses. Four samples were excluded from molecular testing due to either being female and older than 50 years or being male. Subsequently, final analyses had 102 patients for consideration. Patient age, race, and residence zip codes were abstracted from the electronic medical record system, Epic Hyperspace (Epic Systems). To determine relative proximity to the Henry Ford Hospital in Detroit, each patient's zip code was abstracted and entered into MapQuest along with Henry Ford Hospital's zip code. The resulting distance was recorded in miles. All analyses were conducted with deidentified data. A spreadsheet application was used for capturing, presenting, and calculating the data.
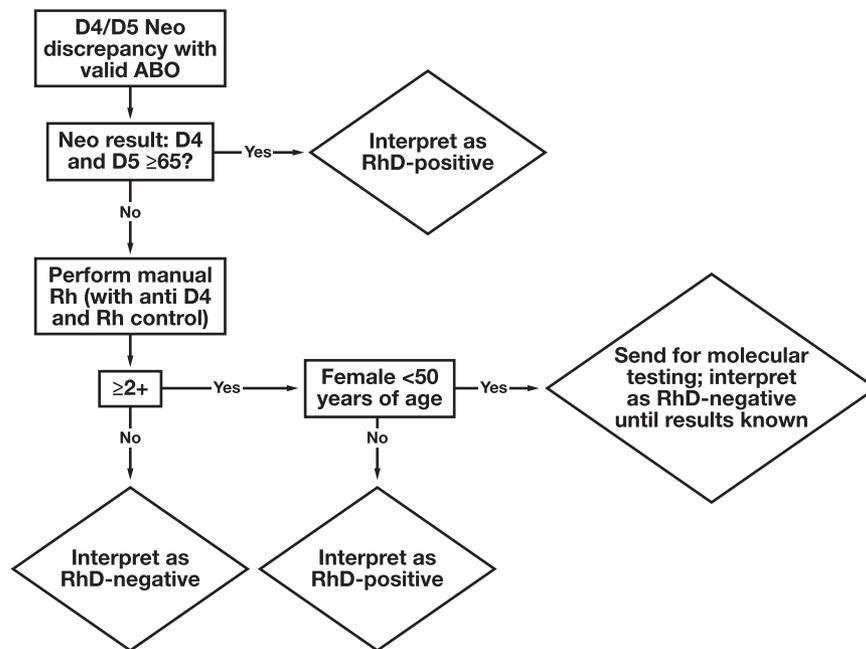
**Figure 1** RhD and molecular determination process used at Henry Ford Health System Blood Bank.

## Molecular Analysis

Molecular analysis was conducted at 2 reference laboratories: Versiti Blood Center of Wisconsin and the American Red Cross in Detroit. Molecular testing was performed using end-point fluorescence detection based on sequence-specific amplification polymerase chain reaction. This assay detected the most common weak D alleles: type 1, 1.1, 2, 3, 4, 4.0, 4.1, 4.2 (DAR), 5.11, 14, 15, and 17 as well as the 3 most common DEL alleles: DEL (M2951), DEL (K409K), and DEL (IVS3 + IG > A). In addition, this assay also detected the most frequent partial D alleles: DIIIa, DIIIb, DIIIc, DIVa, DIVb, DIVa type 3, DIV type 4, DV, DV type 2, DV type 5, DBS-1, DBS-2, DCS, DVI type 1, DVI type 2, DVI type 3, DVI type 4, DVII, DAR, DAU0, DAU1, DAU2/DAU6, DAU3/DAU11, DAU4, DAU5, DAU7, DAU 8-10 and 12-15, DBT type 1, DBT type 2, DFR, DHMi, DHAR, and DNB as well as nonfunctional *RHD* alleles. Per molecular laboratory recommendations during 2016 to 2018, patients with weak D allele 4.2(DAR) and weak D allele type 4.0/4.1 were nominated as RhD negative.

## Results

### Molecular Analysis

Molecular analyses yielded conclusive results in cases with serologic discrepancies. In total, 13 of 102 (12.7%) patients were characterized as RhD positive and 89 of 102 (87.3%) patients were characterized as RhD variants (nominated as RhD negative for practical purposes).

Among those that were classified as weak D, the following alleles, with their respective frequencies, were identified: 4 (3.9%) type 3, 0 (0.0%) type 2, 6 (5.9%) type 1, 1 (0.9%) Rh gene detected, 2 (2.0%) no partial D detected, 11 (10.8%) Rh*weak partial 4.0, 43 (42.2%) type 4.0/4.1, and 5 (4.9%) type 4.2 (DAR). Among those that were classified as partial D, the following alleles, with their respective frequencies, were identified: 3 (2.9%) RHD*weak partial D 4.0/RHD*Ψ, 3 (2.9%) DVII, 3 (2.9%) partial DAU-4 or 5, 6 (5.9%) unknown weak/partial D type, and 15 (14.7%) other partial D allele. **Table 1** illustrates the Rh genotype, partial or weak D classification per the molecular laboratory test results, number of patients (and percentage of total n = 102), and the institutional nomination of RhD negative or RhD positive for practical considerations.
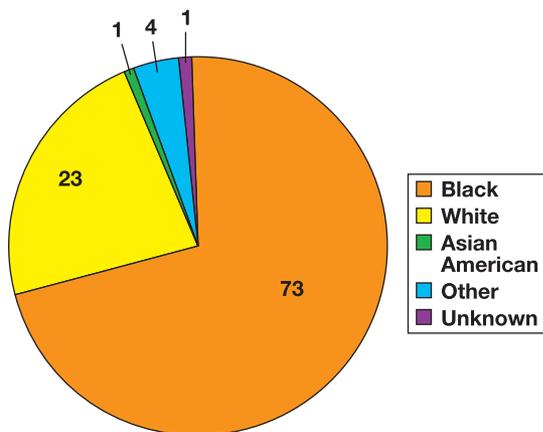
### Racial Classification Analysis

Among this cohort, patients self-identified as 73 (71.5%) Black, 23 (22.5%) White, 1 (0.9%) Asian American, 4 (3.9%) other, and 1 (0.9%) unknown. **Figure 2** presents the racial characteristics and respective frequencies identified in this patient cohort.

In the RhD-variant classification (RhD-negative nomination), there were 69 (67.6%) Blacks, 15 (14.7%) Whites, 1 (0.9%) Asian American, 3 (2.9%) other, and 1 (0.9%) unknown. In the RhD-positive classification, there were 4 (3.9%) Blacks, 8 (7.8%) Whites, 0 (0.0%) Asian Americans, 1 (0.9%) other, and 0 (0.0%) unknown.

**Table 1**

**Rh Genotype Data**

| Rh Genotype | Partial D or Weak D Classification | No. of Patients (%) | Institutional RhD Nomination |
|---|---|---|---|
| Type 1 | Weak D | 6 (5.9) | RhD positive |
| Type 2 | Weak D | 0 (0.0) | RhD positive |
| Type 3 | Weak D | 4 (3.9) | RhD positive |
| Rh gene detected | Weak D | 1 (0.9) | RhD positive |
| No partial D detected | Weak D | 2 (2.0) | RhD positive |
| Rh*weak partial 4.0 | Weak D | 11 (10.8) | RhD negative |
| RHD*weak partial D 4.0/RHD*Ψ | Partial D | 3 (2.9) | RhD negative |
| Type 4.0/4.1* | Weak D | 43 (42.2) | RhD negative |
| Type 4.2 (DAR)* | Weak D | 5 (4.9) | RhD negative |
| DVII | Partial D | 3 (2.9) | RhD negative |
| Partial DAU 4 or 5 | Partial D | 3 (2.9) | RhD negative |
| Unknown weak/partial D type | Partial D | 6 (5.9) | RhD negative |
| Other | Partial D | 15 (14.7) | RhD negative |
| Total | | 102 (100) | |



**Figure 2** Self-identified racial classification and numbers of patients in Detroit, Michigan.

Our results demonstrate that a little over half of our cohort lived within 15 miles of Henry Ford Hospital. This is in contrast to the White category where the majority were noted to live greater than 15 miles away from Henry Ford Hospital. **Table 2** presents the patients that identified as Black, their relative proximity to Henry Ford Hospital, and their corresponding RhD nomination. Table 2 demonstrates that the majority of Blacks that were nominated as RhD negative lived within 15 miles of the hospital.

## Discussion

It is important to distinguish between the 3 categories of the D antigen because the risk of alloimmunization is more prevalent in certain groups. The D antigen category has been traditionally thought of as a mosaic with various puzzle pieces—or epitopes—present.[1] However, some of those pieces can be missing—this is where we have what is

**Table 2**

**Self-Identified Black Racial Classification, Geographic Proximity to Henry Ford Hospital, Number of Patients, and Percentage of Total Blacks Tested (n = 73)**

| RhD Institutional Nomination | <5 Miles | >5 and <10 Miles | >10 and <15 Miles | >15 Miles |
|---|---|---|---|---|
| RhD negative | 15 (20.5) | 26 (35.6) | 8 (11.0) | 20 (27.4) |
| RhD positive | 0 (0.0) | 3 (4.1) | 0 (0.0) | 1 (1.4) |

Data are No. (%).

considered a partial D.[1] On a molecular level, partial Ds arise from *RHD/CE* hybrid alleles, missense mutations in the extracellular loops, or amino acid substitutions.[13] As a result, when a partial D individual gets transfused with RhD-positive RBC, alloimmunization can occur because the individual can produce a D-antibody to the epitope(s) of the D antigen mosaic they lack.[1] Common partial D categories include DII to DVII, DBT, DFR, DHAR, and DAU-5, among others.[1] The historic understanding has been that partial Ds can produce an antibody response, while those with a weak D cannot; however, this interpretation does not hold true anymore.[1]

Weak D antigens are more complex to define as their definition regarding epitopes is not as straightforward.[1] From a molecular standpoint, weak Ds can arise from missense mutations.[13] The overarching theme throughout transfusion history, however, has been that most weak D antigens have a normal D antigen.[14-16] This has prompted clinicians to disregard the possibility of alloimmunization in weak D patients.[16,17] However, weak Ds need to be interpreted with caution as it has now been proven that there are some weak D antigens that have been associated with an allo-anti-D.[4] For example, DAR types and types 4.2, 11, 15, 21, and 57 are under the weak D classification, but they have been associated in patients who have made

an anti-D.[18] Therefore, women of childbearing potential with these types need to be evaluated for RhIG prophylaxis. Similarly, weak D type 4.0 has also been known to cause anti-D alloimmunization in some patients.[19,20]

Additionally, the D antigen has been proven to be one of the most immunogenic blood group antigens.[21] Its implications in fetal-maternal medicine were first proven by Chown[22] by showcasing that fetal RBCs could cross the placenta and enter maternal circulation, which could consequently cause a fetal-maternal hemorrhage. In the case of an RhD-incompatible pregnancy, with the mother being RhD negative and the baby RhD positive, the fetal maternal hemorrhage could cause alloimmunization in the mother, which could potentially lead to HDFN in the current or any subsequent pregnancy.

In developed countries, to reduce the risk of HDFN, the administration of antepartum and postpartum RhIG to RhD-negative mothers has become standard practice. Overall, RhIG has proven to be successful in reducing the risk of HDFN.[23] A meta-analysis performed by Jones et al[23] concluded that in 2 United Kingdom nonrandomized, community studies, the risk of sensitization had an associated odds ratio of 0.37 and an absolute reduced risk to 0.6% for RhD-negative mothers carrying RhD-positive children. More recently, it has been proven that the administration of RhIG in first pregnancies reduces the risk of sensitization to approximately 0.2%.[24]

Challenges, however, arise when RhD variants are introduced. Currently, serologic techniques do not allow us to distinguish between weak D and partial D; molecular assays are the only method that allows this distinction between weak D or partial D.[3] Furthermore, even that distinction is not enough as it has been shown that there are certain weak Ds that can produce an allo-anti-D; therefore, specific allele types have to be known.[4] Earlier studies have shown that approximately 90% of Europeans with a serologic weak D have weak D types 1 to 4.[25] Therefore, at the time, the authors concluded that these individuals could be safely transfused with RhD-positive RBCs and were not candidates for RhIG prophylaxis.[25] However, there has been a slight change to this in recent years. There have been many studies that recommend treating weak D type 4.0 as RhD negative and administering RhIG prophylaxis.[4,18,26] Nonetheless, this is not consistent with our results because our population is not a predominantly European population. A study conducted by Schulz et al[27] found that the racial composition of Detroit was 57% Black, 22% Latino, and 19% White. In our Detroit cohort, approximately 12.7% were classified as weak D and RhD positive. Therefore, this paradigm where the molecular results are based on European cohorts cannot be applied to populations of mixed origin or populations that are predominantly Black.

Various studies have uncovered racial and ethnic patterns in various blood group antigens and specifically the D antigen.[28,29] Dezan et al[28] specifically found that the prevalence of partial D among individuals of mixed origin—a mixture of European and African decent—was high. Knowing that Detroit's racial and ethnic composition does not fit the European cohorts that are prevalent in most research studies, we decided to quantify the prevalence of variant RhDs in this patient population so we could learn the needs of our community and provide population-specific care to serve them better. Our cohort produced 73 (71.5%) patients that identified as Black and 23 (22.5%) patients that identified as White. Furthermore, we were able to find that 69 Black patients (93.2% of patients in the Black category) were RhD variants (RhD-negative nomination) after molecular results. Overall, taking our entire cohort into account, 89 (87.3%) were candidates for RhIG prophylaxis.

Our study was consistent with a research study conducted by Bub et al[30] in which the researchers had a multiethnic cohort that was a mix of European, African, and Native American in their ethnic background. In their cohort of 104 patients with D antigen serologic discrepancies, they found that 22% of pregnant women were not at risk for producing anti-D while 78% of pregnant women were at risk (definitive and potential) for producing anti-D and were candidates for RhIG.[30] Furthermore, we looked at the number of patients that had weak D alleles that needed to be treated as RhD negative: types 4.0/4.1 and 4.2 (DAR). We further analyzed these 2 alleles among Blacks ❚Figure 3❚. We found that a high proportion of Blacks (34 out of 43 total) tested positive for type 4.0/4.1. This further supports our theory that women of childbearing potential in Black communities, like Detroit, are at an
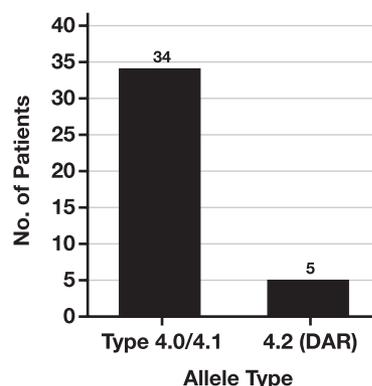


❚Figure 3❚ Numbers of weak D allele types that need to be treated as RhD-negative among Black patients.

increased risk for having serologic D antigen discrepancies that are consistent with potential alloimmunization and HDFN.

To understand our community better, we also looked at geographic proximity of our patients in relation to Henry Ford Hospital. Our study concluded that the majority of these patients (50.9%) lived within 15 miles of Henry Ford Hospital. We also looked at the Black population and their relative proximity to Henry Ford Hospital. We found that 56.1% that had a serologic discrepancy that would need to be classified as RhD variant with the potential of an allo-anti-D lived within 10 miles of the hospital. Therefore, this gives us the opportunity to understand the needs of our community better and raise awareness among medical practitioners in regard to the patients they treat. By extension, this not only allows us to educate medical practitioners but also educate our patient population of the increased risk they face of a serologic D discrepancy. Furthermore, this study started off as a pilot program at our institution but is now part of the standard operating procedure at the Henry Ford Health System.

There are a certain number of limitations to this study that have to be considered. There is a wide array of what constitutes an African ancestry and there are a number of individuals who are considered mixed in origin that have various ethnic backgrounds, including African ancestry. Therefore, the results of this study would be best applicable to racial and ethnic compositions that are similar to those in Detroit, Michigan. Even though our medical record system has broad racial categories, the options are still not specific enough to encompass the racial and ethnic uniqueness of individuals. Larger studies are needed to validate our findings along with different ethnic and racial compositions that have more precise categories. The limitations of genotyping as a practice have to also be considered. It is an expensive service with a long turnaround time. As a result, accessibility is an issue for some smaller laboratories in the United States or other countries in limited resource settings.

In conclusion, this cross-sectional study gives clinical relevance and insight into the diversity of the D antigen among populations that do not identify as White. We have shown that approximately 12.7% of our Black majority cohort classified as RhD positive and 87.3% classified as RhD variants (RhD-negative nomination at our institution). These results can facilitate the proper management of obstetric populations in this patient population. Lastly, the proper RhD-negative or RhD-positive treatment of Rh variants goes beyond the preservation of RhD-negative RBCs; it can help mitigate the potential adverse fetal maternal outcomes.

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