

BloodCenter of Wisconsin offers genotyping tests for Weak RHD alleles.

BACKGROUND:

The Rh D polypeptide is a highly immunogenic protein present on the red cell surface of approximately 85% of Caucasians, >90% of Africans and nearly 100% of Asians. Some of the more than 200 RHD alleles lead to a reduced or variable expression of D antigenic epitopes on the red cell surface.¹ Patients with these aberrant alleles may be mistyped by serology because many of the alleles do not react equally with all anti-D typing reagents. Most often, discrepancies with historical results are observed when laboratories change methodologies or reagents. It is important to resolve these discrepancies to determine appropriate anti-D prophylaxis for pregnant women and the Rh status for transfusion recipients at risk of making anti-D. In most cases, molecular analyses can be used to identify RHD alleles that can be deemed Rh positive. Presently, Weak D Types 1, 2, and 3 can be deemed Rh positive since clinical data indicate that patients who express these alleles have a very low risk of making anti-D.^{2,3}

The Rh D Discrepancy Analysis is not suitable to characterize suspected partial D alleles (e.g. Rh-positive with anti-D). For these cases, please order 'PARTIAL D ANALYSIS'.

REASONS FOR REFERRAL:

- Resolution of a prenatal Rh D typing discrepancy.
- Resolution of a pre-transfusion Rh D typing discrepancy.

METHOD:

1. D Antigen Typing: Four FDA licensed anti-D reagents are used to evaluate the red cell phenotypic expression by direct agglutination, and the indirect antiglobulin test (IAT) for the weak expression of D when necessary.
2. Weak D Analysis: DNA is isolated from white cells and allele-specific amplification is performed by PCR to identify RHD polymorphic regions leading to the expression of Weak D Types 1, 2, 3, 4.0, 5, 11, 15, 17 DAR and RHD(M295I).³ The amplified products are then evaluated by gel electrophoresis.

LIMITATIONS:

New variant alleles that possess polymorphisms within the region targeted by the oligonucleotide primers may not be identified with this assay.

SPECIMEN REQUIREMENTS:

5 ml EDTA anticoagulated blood collected and shipped at room temperature.

SHIPPING REQUIREMENTS:

Place the specimen and the test requisition form into plastic bags and seal. Insert into a Styrofoam container; seal and place into a sturdy cardboard box, tape securely and ship by an overnight carrier. Ship the package in compliance with your overnight carrier guidelines. Please notify the laboratory if shipping on Friday, Saturday or the day before a holiday. (Call 800-245-3117, ext. 6250). Label with the following address:

Client Services/Immunohematology Reference Laboratory
BloodCenter of Wisconsin
638 North 18th Street
Milwaukee, WI 53233
800-245-3117 x6250

TURNAROUND TIME: 2 days

CPT CODES:

D Antigen Typing: 86901x4

Weak D Analysis: 81479

REFERENCES:

1. Flegel WA. Molecular genetics and clinical applications for RH. Transfusion and Apheresis Science 2011;44:81-91.
2. Flegel WA, Denomme GA, Yazer MH. On the complexity of D antigen typing: a handy decision tree in the age of molecular blood group diagnostics. J Obstet Gynaecol Can. 2007;29:746-52.
3. Prager M. Molecular genetic blood group typing by the use of PCR-SSP technique. Transfusion 2007;47: 54S-59S.