Weak RhD Analysis

The RhD 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. Versiti offers testing to identify the most common *RHD* alleles that result in the weak and variable expression of the D antigen. Testing is appropriate to resolve a weak D phenotype or when a historical phenotype does not match current RhD phenotype.

Some of the more than 500 RHD alleles lead to a reduced and variable expression of D antigenic epitopes on the red cell surface.[1,2] Patients with these aberrant alleles may be mistyped by serology because many of the alleles do not react equally with all RhD typing reagents.

Most often, discrepancies with historical results are observed when laboratories change methodologies or reagents. It is important to resolve RhD discrepancies to determine appropriate anti-D prophylaxis for pregnant women and the RhD status for transfusion recipients. The Weak RhD Analysis identifies the RHD alleles Weak D Types 1, 1.1, 2, 3, and 4.1 that can be safely deemed RhD Positive since clinical data indicate that patients who express these alleles have a very low risk of making anti-D.[4,5] The analysis uses 'TaqMan' hydrolysis chemistry. Sequence-specific amplification primers are PCR amplified using a 5' nuclease (Thermus aquaticus) thermostable polymerase. Fluorescently labeled oligonucleotide probes are hydrolyzed by the polymerase when the targeted nucleotide is present.

Weak RhD Analysis is seldom suitable to characterize RHD alleles (e.g. partial DVI phenotype) with anti-D detected in the serum or to determine whether a patient is RhD positive or negative. For these cases, the Partial RhD Analysis is often more informative.

Indications for testing:

- Resolution of a prenatal RhD typing discrepancy
- Resolution of a pre-transfusion RhD typing discrepancy

Tests:

1. **RhD Antigen Typing:** Four FDA-licensed anti-D reagents are used to evaluate the red cell phenotypic expression using direct hemagglutination and the indirect antiglobulin test (IAT) for the weak expression of D.

   NOTE: The reference lab may contact the client to recommend Partial RhD Analysis depending on the RhD antigen typing results.

2. **Weak RhD Analysis:** DNA is isolated from white cells and a molecular assay to identify RHD nucleotide single nucleotide variations associated with Weak D Types 1, 1.1, 2, 3, 4.0, 4.1, 4.2 (DAR), 5, 11, 14, 15, 17, and the common D elution (DEL) alleles DEL(M295I), DEL(K409K), and DEL(IVS3+1G>A).

Assay sensitivity and limitations:

Novel and nucleotide variations not targeted by the oligonucleotide amplimers/hydrolysis probes are not detected.

Reporting of results:

1. **Weak D Type 1, 1.1, 2, 3, and 4.1.**

   Females of childbearing potential are reported as RhD Positive and are not candidates for antenatal/postpartum Rh immune globulin prophylaxis. All transfusion recipients are reported as RhD Positive and can receive RhD Positive blood.

2. **Weak D Type 4.0.**

   Our policy is to use an abundance of caution5 and report females of childbearing potential as 'RhD Variant'. They are candidates for antenatal/postpartum Rh immune globulin prophylaxis and should be transfused with RhD Negative blood. Female transfusion recipients over the age of 50 and all male transfusion recipients are reported as RhD Positive and can receive RhD Positive blood.

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3. **Weak D Types 4.2(DAR), 5, 11, 14, 15, 17, DEL(M295I), DEL(K409K), and DEL(IVS3+1G>A).**

   Females of childbearing potential are reported as 'RhD Variant' and are candidates for antenatal/postpartum Rh immune globulin prophylaxis. All transfusion recipients are reported as 'RhD Variant' and should receive RhD Negative blood.

4. **No Weak D Type identified.**

   Females of childbearing potential are reported as 'RhD Variant' and are candidates for antenatal/postpartum Rh immune globulin prophylaxis if the RhD antigen typing is weak or variable. All transfusion recipients are reported as 'RhD Variant' and should receive RhD Negative blood, if suggested by the RhD antigen typing A recommendation to request the Partial RhD Analysis if clinically indicated is included in the report.

Specimen requirements:

5 ml EDTA Whole Blood (lavender top) collected and shipped at ambient temperature.

SHIP

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, option 1).

Label with the following address:

Versiti Wisconsin
Client Services/Immunohematology Reference Lab
638 N. 18th St.
Milwaukee, WI 53233
800-245-3117, ext. 6250

ORDER

Required forms:

Immunohematology Reference Lab

Please complete all pages of the requisition form. Clinical history (including patient's ethnicity, clinical diagnosis, family history and relevant laboratory findings) is necessary for optimal interpretation of genetic test results and recommendations. Clinical and laboratory history can either be recorded on the requisition form or clinical and laboratory reports can be submitted with the sample.

CPT Codes/Billing/Turnaround time:

RhD Antigen Typing: 86901 x 4

Weak RhD Analysis: 81479

Turnaround time: 3-5 days

CPT and Order Codes are provided for reference purposes only and are subject to change. They are not intended as a guide for internal billing procedures. Institution is solely responsible for identification of correct billing codes.

For additional information related to shipping, billing or pricing, please contact, Versiti Client Services: (414) 937-6396 or 800-245-3117, Option 1, or LabInfo@versiti.org.

References:

1. Sandler SG, Chen LN, Flegel WA. Serological weak D phenotypes: a review and guidance for interpreting the RhD blood type using the RHD genotype. Br J Haematol 2017 Oct;179(1):10-19.
2. Sandler SG, Queenan JT. A Guide to Terminology for Rh Immunoprophylaxis. Obstet Gynecol 2017 Sep;130(3):633-635.
3. Virk M, Sandler SG. Rh Immunoprophylaxis for Women with a Serologic Weak D Phenotype. Lab Med 2015;46(3):190-4.
4. Sandler SG, Flegel WA, Westhoff CM, Denomme GA, et al. It's time to phase in RHD genotyping for patients with a serologic weak D phenotype. College of American Pathologists Transfusion Medicine Resource Committee Work Group. Transfusion 2015 Mar;55(3):680-9.
5. Flegel WA, Denomme GA, Queenan JT, Johnson ST, Keller MA, Westhoff CM, Katz LM, Delaney M, Vassallo RR, Simon CD, Sandler SG. It's time to phase out "serologic weak D phenotype" and resolve D types with RHD genotyping including weak D type 4. Transfusion 2020 Apr;60(4):855-859.

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