Canadian Blood Services
BLOOD
PLASMA
STEM CELLS
ORGANS
& TISSUES

Edmonton Diagnostic Services
Immunohematology Referral Testing Services

Test for *RHD* Genotyping (Frequently Asked Questions)
AB_REF-10A

1. Why is it important to provide the clinical and serologic history on the *RHD* genotyping requisition?

Red cell genotyping assays must be interpreted in the context of the patient's clinical history and serologic test results. When sending a sample for *RHD* genotyping please ensure the following information is included on the requisition and attach applicable serologic test results:

- Clinical indication for testing (for example: prenatal or individual of childbearing potential, underlying sickle cell disease or other hemoglobinopathy, chronic transfusion recipient)
- Serologic findings (for example: strength of serologic RhD typing results; results of antibody investigation)
- Clinical history of allogeneic hematopoietic stem cell transplant or underlying hematologic neoplasm
- Recent transfusion history (past three months)

2. How is *RHD* genotyping performed at Canadian Blood Services?

Canadian Blood Services uses the Immucor *RHD* Molecular BeadChip Test. The test interrogates 35 genetic markers to resolve 66 alleles.[1] This targeted multiplex polymerase chain reaction (PCR) based assay is designed to detect the most common *RHD* variants associated with weak D, partial D, Del and D negative phenotypes. It does not involve sequencing of the *RHD* gene. Please see the list of variant *RHD* alleles detected by this test in Appendix A.

3. How many *RHD* variants are there?

The *RHD* gene shows remarkable biodiversity with several hundred unique alleles listed in the International Society of Blood Transfusion (ISBT) allele table and described in the literature.[2] The number of identified *RHD* alleles has increased significantly in recent years with advances in sequencing technology and large population-based studies. The distribution of *RHD* alleles varies across different ethnic and geographic populations. In the context of the ethnically diverse Canadian population, some variant *RHD* alleles will not be identified by a targeted *RHD* genotyping assay.

4. What are the limitations of a targeted *RHD* genotyping assay?

- As part of ongoing development work in red cell genomics at Canadian Blood Services, a recent case review has shown that despite the absence of a detectable *RHD* variant on the assay, a serologic weak D phenotype strongly predicts for the presence of a variant *RHD* allele by gene sequencing.[3]
- Patients with suspected alloanti-D antibodies for whom no *RHD* variants are detected may require additional investigations (including a comprehensive serologic assessment by a reference lab with consideration of referred-out gene sequencing on a case-by-case basis).
- The test cannot assess for dual genetic populations which may be seen in the setting of allogeneic hematopoietic stem cell transplantation or some hematologic malignancies
- The assay cannot assess for *RHD* allele zygosity (it cannot be used to assess if a partner of a pregnant patient with anti-D carries one or two copies of an *RHD* allele)

5. How are the results interpreted and reported?

A recommendation to treat the patient as RhD positive or RhD negative will be provided in the report based on a careful review of the genetic markers, the clinical history and the serologic test results.

If a variant allele is detected, the common name of the allele and the ISBT nomenclature will be reported. The assay cannot distinguish between the hemizygous (variant allele paired with an *RHD* deletion) and homozygous state (two copies of the variant allele).

If both alleles carry an *RHD* gene deletion, the result is reported as *RHD*deletion* (*RHD**01N.01).

In the absence of a detectable variant allele or *RHD* deletion, the test returns a result of "possible D". As per the package insert, this should be interpreted as the absence of *RHD* variants covered in the panel rather than the presence of an unaltered *RHD* gene and is reported as "No RHD variants detected".[1]

For samples referred for serologic weak D phenotype (including variable or historically discordant RhD typing results) with no detectable *RHD* variants, the recommendation is to consider these individuals as RhD negative given the high chance that they carry an *RHD* variant missed by the assay.[3] Each lab must develop an appropriate algorithm for identifying and managing a serologic weak D phenotype.[4]

6. Which variant *RHD* alleles are considered as *RHD* positive and negative?

While anti-D formation in individuals carrying certain *RHD* variants has been documented, the risk of alloimmunization for many *RHD* variants is uncertain.[5-7] The risk of D alloimmunization leading to hemolytic disease of the fetus and newborn (HDFN) and the clinical efficacy of Rh immune globulin (RhIG) prophylaxis in the setting of maternal *RHD* variants have not been well studied in the literature.

Current guidelines err on the side of caution and recommend that patients with *RHD* variants other than weak D type 1, 2, or 3 be managed as RhD negative and be considered eligible for RhIG prophylaxis. Experts are divided as to whether weak D type 4.0 and 4.1 can be considered as RhD positive. In accordance with the recent National Advisory Committee on Blood and Blood Products (NAC), these variants should be considered RhD negative until further data is available.[8,9]

The management of any given patient may depend on their unique clinical circumstances (age, childbearing potential, transfusion requirements, underlying hemoglobinopathy etc) and serologic test results.[10]

7. What is the turnaround time for results of *RHD* genotyping?

Samples for *RHD* genotyping are batched for testing and the turnaround time for an uncomplicated case is approximately 14 days.

8. Who can I talk to about the results of *RHD* genotyping?

For consultation regarding *RHD* genotyping results please contact the Canadian Blood Services Diagnostic Services Laboratory in Edmonton at 780-431-8765 or via email at genotyping.edm@blood.ca.

APPENDIX A: Alleles detected by the *RHD* Molecular BeadChip Test

Common Designation	ISBT Nomenclature	Common Designation	ISBT Nomenclature
Weak D alleles			
weak D type 1	*RHD*01W.1*	weak D type 17	*RHD*01W.17*
weak D type 1.1	*RHD*01W.1.1*	weak D type 29	*RHD*01W.29*
weak D type 2	*RHD*01W.2*	weak D type 34	*RHD*01W.34*
weak D type 3	*RHD*01W.3*	weak D type 41	*RHD*01W.41*
weak D type 5	*RHD*01W.5*	weak D type 47	*RHD*01W.47*
weak D type 14 or 40 or 51	*RHD*01W.14 or RHD*01W.40 or RHD*01W.51*	weak D type 100	*RHD*01W.100*
Partial D alleles			
DIIIa	*RHD*03.01*	weak D type 4.1	*RHD*09.04*
DIIIb	*RHD*03.02*	DAU1	*RHD*10.01*
DIIIc	*RHD*03.03*	DAU2	*RHD*10.02*
DIII type 4	*RHD*03.04*	DAU3	*RHD*10.03*
DIII type 6 or DIII type 7	*RHD*03.06 or RHD*03.07*	DAU4 or DV type 5	*RHD*10.04 or RHD*05.05*
DIII type 7	*RHD*03.07*	DAU5 or DV type 1 or DBS2	*RHD*10.05 or RHD*05.01 or RHD*13.02*
DIVa	*RHD*04.01*	weak D type 11	*RHD*11*
DIV type 3	*RHD*04.03*	DOL or DOL2	*RHD*12.01 or RHD*12.02*
DIV type 4	*RHD*04.04*	DOL3	*RHD*12.03*
DIV type 5 or DIVb	*RHD*04.05 or RHD*04.06*	DBT1	*RHD*14.01*
DIVb	*RHD*04.06*	DBT2	*RHD*14.02*
DV type 2 or DBS1	*RHD*05.02 or RHD*13.01*	weak D type 15	*RHD*15*
DV type 2 or DBS1 or DV type 7	*RHD*05.02 or RHD*13.01 or RHD*05.07*	DCS1 or DFV	*RHD*16.01 or RHD*30*
DBSO	*RHD*05.03*	DCS2	*RHD*16.02*
DV type 4	*RHD*05.04*	DFR or DFR3	*RHD*17.01 or RHD*17.03*
DV type 6	*RHD*05.06*	DFR2	*RHD*17.02*
DV type 8	*RHD*05.08*	DFR4	*RHD*17.04*
DV type 9	*RHD*05.09*	DHMi	*RHD*19*
DVI	*RHD*06*	DNB	*RHD*25*
DAR	*RHD*09.01*	DUC2	*RHD*37*
DAR-E	*RHD*09.02*	ceHAR	*RHCE*ce22.01*
weak D type 4.0 or 4.3	*RHD*09.03 or RHD*09.05*		
Del alleles			
RHD*1227A	*RHD*01.EL.01*	IVS3+1G>A	*RHD*01EL.08*
Null alleles (D Negative)			
RHD deletion	*RHD*01N.01*	RHD*807G (Y269X)	*RHD*01N.18*
RHD-CE(3-9)-D	*RHD*01N.04*	DIIIa-CE(4-7)-D	*RHD*03N.01*
RHD-CE(3-7)-D	*RHD*01N.06*	RHCE(1-3)-D(4-10)	*RHD*01N.43*
RHD-CE(4-7)-D	*RHD*01N.07*	RHD psi (Pseudogene)	*RHD*08N.01*
RHD*48A (W16X)	*RHD*01N.08*		

Notes: 1) The phenotypic classification of the *RHD* alleles (weak, partial, Del, null) is based on the ISBT allele table. The distinction between weak and partial D alleles is not absolute. For example: weak D type 4.0, 11 and 15 are considered weak partial D alleles. 2) The test may detect genetic markers common to two or three *RHD* alleles but is unable to discriminate between these. 3) ceHAR is an *RHCE* allele that may react with some monoclonal anti-D typing reagents.

References:

1. Immucor RHD Molecular BeadChip Test Package Insert (Date of revision: 10-2019)

2. International Society of Blood Transfusion (ISBT) RHD Allele tables v6.3 31-MAR-2023 available at https://www.isbtweb.org/resource/004*RHD*.html

3. Bodnar, M., Clarke, G., Denomme, G., et al., Blindspots in targeted RHD genotyping: a review of cases sent for gene sequencing. Transfusion. 2022; 62,118A (abstract).

4. Ramsey, G., Park, Y., Eder, A., et al., Obstetric and newborn weak D-phenotype RBC Testing and Rh Immune Globulin management recommendations: Lessons from a blinded specimen-testing survey of 81 transfusion services. Arch Pathol Lab Med. 2023; 147(1): 71-78. (available at https://meridian.allenpress.com/aplm/article/147/1/71/480973/Obstetric-and-Newborn-Weak-D-Phenotype-RBC-Testing)

5. Floch A, Téletchéa S, Tournamille C, de Brevern AG, Pirenne F. A review of the literature organized into a new database: RHeference. Transfusion Medicine Reviews. 2021; 35 (2): 70-77. Available at https://www.rheference.org/

6. Floch, A. Molecular genetics of the Rh blood group system: alleles and antibodies—a narrative review. Ann Blood. 2021; 6:29. Available at https://aob.amegroups.org/article/view/6532/html

7. Takasaki, K., Friedman, D., Uter, S., et al., Variant RHD alleles and Rh immunization in patients with sickle cell disease. British Journal of Haematology. 2023; 201(6) 1220-1228.

8. Flegel, W., Denomme, G., Queenan, J., et al., It's time to phase out "serologic weak D phenotype" and resolve D types with RHD genotyping including weak D type 4. Transfusion. 2020; 60(4) 855-859.

9. National Advisory Committee on Blood and Blood Products (NAC) RHD genotyping in Prenatal Patients (May 2022) available at https://nacblood.ca/en/resource/*RHD*-genotyping-prenatal-patients

10. Daniels, G., Variants of RHD – current testing and clinical consequences. British Journal of Haematology. 2013; 161, 461-470.