



Government of Newfoundland and Labrador

Department of Health and Community Services
Provincial Blood Coordinating Program

RH D AND WEAK D TESTING	NLBCP-009
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Overview

Rhesus (Rh) factor, RhD, is an inherited protein found on the surface of red blood cells (RBCs). The RhD antigen contains over 30 epitopes, which are the part of an antigen that is recognized by the immune system. RhD status is usually positive or negative, however, in some situations variations of the D phenotype arise when certain D epitopes are only weakly expressed ("weak D phenotype") or when some are missing ("partial D phenotype").

Policy

1. Each recipient blood sample received for pre-transfusion testing shall be tested for RhD.
2. Recipient RBCs shall be tested with anti-D reagent. An RhD control system appropriate to the anti-D reagent in use must be included. RhD control shall be negative for valid RhD type.
3. The Medical Laboratory Technologist (MLT) shall check the prenatal status of the patient being tested.
4. A test for weak D is not always required with the RhD typing.

Weak D testing shall be performed in the case of:

- 4.1. An RhD negative neonate of an RhD negative birthing parent (to determine risk of D immunization); and
- 4.2. To resolve discrepancies between current and previous results.
5. Weak D testing shall not be performed on samples in which the Direct Antiglobulin Test (DAT) is positive, unless there is a mechanism used to desensitize the RBCs to perform weak D testing.
6. All adult and pediatric samples that test RhD negative or less than or equal to 2+ on initial testing shall be confirmed RhD negative using a broad reactivity anti-D reagent.
7. Positive results with broad reactivity anti-D reagent shall be evaluated based on Newfoundland and Labrador Health Services (NLHS) established policy.
8. Current and previous results should be compared to identify any discrepancy. All discrepancies shall be resolved and the resolution documented before reporting.
9. If an RhD typing discrepancy is detected or in an emergency situation:
 - 9.1. RhD negative recipients should be transfused with RhD negative RBCs. Follow NLHS established policy for the transfusion of RhD positive RBCs to an RhD negative recipient when RhD negative RBCs are in diminished supply.
 - 9.2. RhD negative individuals of child-bearing potential and children shall receive RhD negative RBCs, except for a life-threatening situation and when RhD negative RBCs are not available.

10. RhD positive recipients may receive RBCs that are either RhD positive or RhD negative.
11. NLHS shall have a policy for the administration of Rh immune globulin (Rhlg) for RhD negative recipients who receive blood components containing RhD positive RBCs.
12. All individuals of child-bearing potential undergoing delivery, abortion, or invasive obstetric procedures shall have their RhD group determined. A policy shall be in place regarding the administration of Rhlg to RhD negative individuals of child-bearing potential who are tested to be weak D or partial D.
13. There shall be criteria for interpreting RhD grouping results that will prevent the misgrouping of an RhD negative birthing parent as RhD positive.
14. Individuals of child-bearing potential with less than or equal to 2+ grade reaction with limited specificity anti-D reagent may be considered for genotyping, based on clinical picture.
15. While prenatal samples are sent for genotyping, decision to refer any other sample types shall be determined on a case-by-case basis by the Transfusion Medicine Medical Director or designate.

Guidelines

1. Due to changes in the RhD reporting process, there may be occasions when patients were previously reported as RhD positive and must now be reported as RhD negative. A second sample may not be required to confirm this change because it is a result of an update in policy. The attached physician memo may be sent to the attending physician to assist in explaining the change in patients' blood group (see Appendix D).
2. If two limited specificity anti-D reagents are used for initial testing, the strengths should agree within two grades. If strengths do not agree, the patient should be investigated.
3. Very weak reactions may indicate the presence of quantitatively weak D or partial D antigen.
4. Anti-D reagents may not detect all examples of partial D. Reagents may react with weak D cells and rare examples of partial D cells that may have previously been tested and interpreted as RhD negative using other sources of Anti-D.
5. Interpreting positive weak D testing to determine if RhD negative birth parent is a Rhlg candidate:
 - 5.1. Paternal specimens: This individual should be considered RhD positive. RhD negative birth parent is a Rhlg candidate.
 - 5.2. Neonatal specimens: This neonate should be considered RhD positive. RhD negative birth parent is a Rhlg candidate. For the purposes of transfusion, this neonate should be considered RhD negative.

- 5.3. Cord specimens: This sample should be considered RhD positive. RhD negative birth parent is a RhIg candidate. For the purposes of transfusion, this neonate should be considered RhD negative.
- 5.4. Positive DAT: Unable to report weak D test due to positive DAT. RhD negative birth parent is a RhIg candidate.
6. RhD genotyping is useful in distinguishing partial D from weak D or to resolve RhD typing discrepancies. This is most beneficial in individuals of child-bearing potential who may require RhIg.

Quality Control

All reagents shall be used and controlled according to the manufacturer product insert.

Key Words

RhD, RhIg, Weak D, Partial D, Rh typing

Supplemental Materials

Appendix A Interpretation of weak D Phenotyping

Appendix B Interpretation of All Samples Except Prenatal

Appendix C Interpretation of Prenatal Samples

Appendix D Physician Memo for RhD Testing and Reporting

References

Canadian Standards Association. (2020). *Blood and blood components, Z902-20, Amendments*. Mississauga (ON): Author.

Canadian Society for Transfusion Medicine. (2022). *Standards for Hospital Transfusion Services*. (Version 5.0). Markham, ON: Author.

Denomme, G.A. *The Rh System*. In: Fung M, Eder, A.F., Spitalnik, S.L, & Westhoff CM. Technical manual. 19th edition. Bethesda, MD: AABB, 2017:295-314.

Nova Scotia Provincial Blood Coordinating Team. (2022). *Guideline for Rh Testing in Nova Scotia* (Version 3.0). Halifax, NS: Author.

Appendix A

Interpretation of weak D Phenotyping

Weak D Test Results		Interpretation
ANTI-D	CONTROL	
Neg	Neg	RhD negative
Pos	Neg	<p>RhD positive</p> <p>A canned text may be reported along with this result stating:</p> <p>This patient sample exhibits a weakened or partial expression of the D antigen. Birthing partent is a candidate to receive RhIg if not already administered.</p> <p>For transfusion purposes the patient will be considered RhD negative. Future testing may indicate a change in RhD status from RhD negative to RhD positive.</p>
Pos	Pos	<p>Invalid results</p> <p>Further investigation is required.</p>

Appendix B

Interpretation of All Samples Except Prenatal

Limited Specificity Anti-D (routine testing)	Broad Reactivity Anti-D	Interpretation
4+	Not required	RhD positive
3+	Not required	RhD positive
Less than or equal to 2+ or negative on initial testing of patient (no patient history)	Any positive grade after five-minute room temperature incubation	RhD Indeterminate *Investigate according to NLHS policy.
Negative	Negative	RhD negative

Appendix C

Interpretation of Prenatal Samples

Limited Specificity Anti-D (routine testing)	Broad Reactivity Anti-D	Interpretation
4+	Not required	RhD positive
3+	Not required	RhD positive
Less than or equal to 2+ or negative on initial testing of patient (no patient history)	4+, 3+ after five-minute room temperature incubation	RhD positive
Less than or equal to 2+ or negative on initial testing of patient (no patient history)	2+, 1+ or weak after a five-minute room temperature incubation	<p>The RhD typing should not be reported. Samples should be collected to send to the National Reference Laboratory for genotyping.</p> <p>The patients ABO blood group can be reported in the LIS along with a comment or “canned text” stating:</p> <p>RhD status is indeterminate due to a suspected weak or partial expression of the D antigen. Additional samples are required for RhD genotyping. This testing is performed by the Canadian Blood Services National Immunohematology Reference Laboratory (NIRL).</p> <p>The patient will be considered as RhD negative with respect to transfusion and Rh prophylaxis until genotyping is complete.</p>
Negative	Negative	RhD negative

Appendix D

Physician Memo for RhD Testing and Reporting

The Newfoundland and Labrador Provincial Blood Coordinating Program has developed a best practice provincial approach to RhD testing and reporting for weak D and partial D Rhesus antigens that may result in:

1. Certain patients, who were previously grouped as RhD positive, will be reported as RhD negative in the future or vice versa.
2. Certain prenatal patients will be reported as Rh indeterminate pending further testing.

Individuals presenting with weak D have been historically defined as having a reduced amount of D antigen on the surface of their red blood cells, while those exhibiting partial D have a structurally altered D antigen.

RhD genotyping is useful in distinguishing partial D from weak D or to resolve RhD typing discrepancies. This is most beneficial in individuals of child-bearing potential who may require RhD immune globulin. Although the percentage of the population exhibiting these genotypes is low, proper determination allows health care professionals to administer Rh immune globulin only to those who are truly able to produce anti-D antibodies thus eliminating unnecessary exposure to RhD immune globulin.

It is the responsibility of the ordering physician to indicate on the requisition for blood work the patient diagnosis as **prenatal** to ensure the appropriate testing is completed. If discrepant results are obtained, RhD genotyping may be required.

RhD genotyping will only be performed on prenatal patient samples and individuals of child-bearing potential. Rh typing results will be reported as:

'RhD status is indeterminate due to a suspected weak or partial expression of the D antigen. Additional samples are required for RhD genotyping. This testing is performed by Brampton Diagnostic Services. The patient will be considered as RhD negative with respect to transfusion and Rh prophylaxis until genotyping is complete.'

Weak and partial D antigens for all other patient populations will result in the patient being reported as RhD negative. This is a best practice transfusion initiative supported by most, if not all, blood programs in Canada.

Questions related to this initiative may be directed to the Transfusion Medicine Laboratory.