Nova Scotia Provincial Blood Coordinating Team

Guideline for RhD Testing In Nova Scotia

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Version 3.0

PROMOTING EXCELLENCE IN TRANSFUSION MEDICINE
www.gov.ns.ca/health/nspbcp

Developed by the members of the RhD Testing Atlantic Working Group (Appendix E).
The Nova Scotia Provincial Blood Coordinating Team wishes to recognize all previous working group members for their contributions towards the implementation of standardized Rh testing in Nova Scotia

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1. Background

In 2015, AABB and the College of American Pathologists (CAP) assembled an International Working Group to develop recommendations to clarify clinical issues related to RhD typing. The Working Group was organized in response to a CAP survey that identified a lack of standardization concerning laboratory testing and interpretation of Rh blood type in patients with a weak D phenotype and with the administration of Rh immune globulin (Sandler, et al., 2015). The Working Group's final report provides recommendations for RhD genotyping and interpretation for patients with a serological weak D phenotype.

Provincial standardization of Rh testing was a key objective during the development of version 1.0 of this guideline. As a result of its implementation Rh testing and interpretation of Rh status has been standardized throughout Nova Scotia. The discrepancies revealed from the 2014 CAP survey appear to be of greater significance in regions that lack such standard practices (Sandler, Roseff, Domen, Shaz, & Gottschall, 2014). Version 3.0 of the Nova Scotia Provincial Blood Coordinating Team's (NSPBCT) Guideline for RhD Testing has been revised to incorporate recommendations from AABB and CAP and to reflect current standards and best practices in transfusion medicine.

2. Definitions

Broad specificity RhD antisera – polyclonal or monoclonal blend of serological reagent containing a mixture of a large numbers of different antibodies each recognizing different D epitopes (Flegel, Denomme, & Yazer, 2007).

Limited specificity RhD antisera - serological reagent composed of a single monoclonal D antibody or of a blend of anti-Ds each recognizing a different D epitope expressed on selected D variants (Flegel, Denomme, & Yazer, 2007).

RhD genotyping – molecular analysis for the genetic basis of the D antigen. The test is used to provide RhD status when it cannot be conclusively determined due to discrepant, weak or inconclusive serological RhD testing (Goldman, Hannaford, Hannon, Berardi, & Philip, 2016).

Rh Indeterminate – patients who are serological weak D positive and/or whose Rh genotyping results are unknown.

Rh Immunoglobulin (RhIg) - blood product consisting primarily of IgG Anti-D, prepared from pooled human plasma and is effective in the prevention of active RhD alloimmunization (Fung Kee Fung & Eason, 2018).

Serological weak D phenotype – reactivity of RBCs with an RhD antisera giving no or weak (≤2+) reactivity in initial testing but agglutinating moderately or strongly with antihuman globulin (Sandler, et al., 2015).

3. Introduction

The Rh blood group system is the most clinically important of the 36 systems, after ABO (Sandler, Chen, & Flegel, 2017). The D antigen (RhD) is the most immunogenic of all the antigens in the Rh system (Sandler, Chen, & Flegel, 2017). A significant proportion of individuals lack the RhD antigen on their red cells and are able to become sensitized and produce D antibodies if exposed to the RhD antigen. This places RhD negative individuals at risk of hemolytic transfusion reactions and RhD positive fetuses and newborns of RhD negative mothers at risk of hemolytic disease of the fetus and newborn (HDFN) (Sandler, Chen, & Flegel, 2017; Daniels, 2013). Prevention of alloimmunization requires accurately identifying RhD negative individuals and providing RhD negative blood components and prophylactic RhIg treatment during pregnancy. Routine transfusion practice involves RhD testing to ensure RhD negative individuals are identified and managed appropriately.

Most RhD positive red cells show clear-cut positive reactivity after centrifugation with RhD antisera and can be readily classified as RhD positive. However, variable reactivity or discrepant results may be detected in small proportions of patients. If an individual's red cells have weaker than expected reactivity or differing results with alternative reagents or testing methods their red cells may be expressing a variant form of the RhD antigen (Daniels, 2013; Clarke G. , et al., 2016). Further molecular based testing is required before an RhD positive or negative status can be determined.

Molecular RhD genotyping determines the molecular genetic basis for an individual's RhD antigen expression and is used to definitively classify D variants as either Weak D or Partial D. The weak D variant results from alterations in the *RHD* gene that result in one or more amino acid substitutions in the transmembrane or intracellular regions of the RhD antigen (Flegel, Denomme, & Yazer, 2007). This mutation leads to a **decreased** expression of the RhD antigen on the red blood cell membrane (Flegel, Denomme, & Yazer, 2007). Most weak D genotypes in the Caucasian population can be defined as type 1, 2 or 3 (Sandler, et al., 2015; Sandler, Roseff, Domen, Shaz, & Gottschall, 2014; Flegel, Denomme, & Yazer, 2007). These individuals do not form anti-D and thus can be managed safely as RhD positive, eliminating the need for RhIg administration or Rh negative blood components (Sandler, et al., 2015; Denomme, Wagner, Fernandes, Li, & Flegel, 2005).

Partial D genotypes have one or more amino acid substitutions in the extracellular regions of the RhD antigen (Flegel, Denomme, & Yazer, 2007). This mutation results in a **qualitative** change (loss of one or more D epitopes) in the RhD antigen rather than decreased antigen expression (Flegel, Denomme, & Yazer, 2007). Depending on which epitope is altered, partial D individuals may have very strong reactivity with routine RhD testing methods and inadvertently be interpreted as RhD positive (Sandler, Chen, & Flegel, 2017;Daniels, 2013). Partial D individuals may produce anti-D, necessitating a means by which these patients can be identified to ensure RhIg is provided when needed and RhD negative red cells are transfused when required.

CAP and AABB developed recommendations for the management of individuals with a serological weak D phenotype. They advise that RhD genotyping be phased in for patients with a serologic weak D phenotype, and that patients with weak D type 1, 2 or 3 be considered Rh positive for the purposes of RhIg prophylaxis and transfusion (Sandler, et al., 2015). In response to this recommendation the National Advisory Committee on Blood Products (NAC) released the following guidance statement "*prenatal patients with discrepant, weak, or inconclusive serological*

RhD test results should be further investigated with RhD genotyping to determine RhIg candidacy and optimal Rh type for transfusion (National Advisory Committee on Blood and Blood Products, 2017)." This recommendation avoids unnecessary administration of RhIg in pregnant women and increases the availability of RhD negative red blood cells (Sandler, Roseff, Domen, Shaz, & Gottschall, 2014).

The Nova Scotia Provincial Blood Coordinating Team supports the use of RhD genotyping for patients with a serological weak D phenotype and encourages safer transfusion practices and improving red blood cell utilization.

4. RhD Testing

To identify potential RhD variants, a minimum of two different D antisera (one of limited specificity and the second of broad specificity) are required when RhD testing is performed. A two-reagent approach allows discrepancies between different D antisera to be observed (Denomme, Wagner, Fernandes, Li, & Flegel, 2005). Weak or discrepant reactions indicate that an RhD variant may be present and necessitates follow-up RhD genotyping for select patients.

Limited specificity reagents contain anti-D directed against specific epitopes of the RhD antigen and are designed not to react with partial RhD variants thus increasing the probability that partial D individuals will type as RhD negative. Broad specificity reagents are polyclonal or monoclonal blends of anti-D designed to detect many different RhD epitopes and increase the probability of detecting both weak and partial RhD variants. For a list of commonly used commercially available D antisera in Canada and their specificity see Appendix A.

The approach for RhD typing in prenatal, non-prenatal, neonatal and cord specimens differs slightly. When a request for RhD typing is received, the "RhD Typing Flowchart (Appendix B) should be consulted to determine the proper testing strategy. All individuals less than or equal to 45 years of age with childbearing potential require a one-time RhD typing using a minimum of two different D antisera, as described above. Upon completion of the two D typing method, the patient's RhD status is confirmed and subsequent RhD testing can be performed with any D antisera/testing method. Samples from individuals without childbearing potential do not routinely require two D antisera confirmatory testing and can be completed using one D antisera.

Samples from individuals of childbearing potential that have weaker than expected (≤ 2+) reactivity or a discrepancy with historical RhD typing are to be forwarded to Canadian Blood Services (CBS) for RhD genotyping. Initial discrepant, weak, or inconclusive (≤ 2+) serological RhD testing results on individuals without childbearing potential may be brought to the attention of the transfusion medical director. For patients requiring chronic transfusions there is an added benefit to confirming their RhD status using two different D antisera and, if necessary, RhD genotyping. In these circumstances a case-by-case evaluation should be made on the most appropriate course of action.

Patients referred for RhD genotyping will be reported as RhD Indeterminate until results are available, with the exception of Central Zone which will report RhD negative, until genotyping results are received. The Rh Indeterminate status gives physicians and clinical care workers the ability to understand that the patients' RhD status is unknown and further testing is required. Although the patient will be temporarily reported as indeterminate, if they require a transfusion

they will be treated as RhD negative to take the necessary precautions and eliminate the possibility of producing D antibodies.

Standardization of prophylactic RhIg administration during and after the pregnancy requires accurate RhD determination of newborns and/or biological father (White, et al., 2016). For the purpose of maternal RhIg eligibility, paternal and cord or neonate RhD testing must use broad specificity D antisera (Milkins, et al., 2012). As previously described, these reagents increase the probability that individuals with variant RhD antigens will type as RhD positive and thus indicate that an RhD negative mother should receive RhIg. Initial RhD typing results (by immediate spin) with a broad specificity reagent that show no reactivity must be followed by a weak D test (also referred to as RhIAT) to increase the sensitivity of RhD testing. Interpretation of a positive weak D test must be done in correlation with a Direct Antiglobulin Test (DAT). Valid weak D testing cannot be performed on specimens that are DAT positive. In these circumstances, the RhD negative birth parent should be considered a candidate for RhIg prophylaxis.

5. RhD Reporting

A standardized approach to RhD reporting requires the ability of the province's three laboratory information systems (LIS) to:

a) *Flag/halt result transmissions from automated instruments for specimens that require RhD testing with two D antisera, ensuring further testing required is complete before the ABO and RhD group are reported.*

b) *Allow the technologist to identify patients that have been tested with two anti-D reagents and those who have not.*

c) *Result RhD typing for patients with a serological weak D phenotype as Rh Indeterminate when a sample is sent for genotyping.*

For individuals less than or equal to 45 years of age with childbearing potential, the following comment is to be attached to an RhD Indeterminate result. This will help ensure clinical staff understand the reason an RhD positive or negative result is not being reported.

a) *RhD status is indeterminate due to presumed weak or partial expression of the D antigen. Until resolved, this individual will be considered Rh NEGATIVE for transfusion and pregnancy purposes and is a candidate for RhIg prophylaxis.*

The following comments are attached to the appropriate RhD genotyping results once obtained:

a) **Weak D Results Type 1 to 3:** Results of genotype testing *to resolve the RhD Indeterminate status have been received. (CBS Specimen # []). The individual is a RhD variant [result]. Individuals with this variant are not at risk of producing allo-anti-D. This individual will be considered Rh POSITIVE for transfusion and pregnancy purposes and is NOT a candidate for RhIg prophylaxis. (Sandler, S.G., et al, 2015)*

b) ***RhD variant other than Weak D Type 1, 2 or 3****: Results of genotype testing to resolve the RhD Indeterminate status have been received. (CBS Specimen # []) The individual is a RhD variant [result]. Individuals with this variant may be at risk of producing allo-anti-D. This individual will be considered Rh NEGATIVE for transfusion and pregnancy purposes and is a candidate for RhIg prophylaxis. (Sandler, S.G., et al, 2015)*

Regarding weak D (RhIAT) testing and maternal RhIg eligibility, the following comments are to be attached to weak D (RhIAT) positive results:

a) ***Paternal specimens****: Sample demonstrates weak expression of the D antigen. For the purposes of paternal testing, this individual should be considered RhD positive. For the purpose of maternal RhIg eligibility, this individual should be considered RhD positive and the RhD negative partner a candidate for RhIg prophylaxis. Note that depending on the type of testing performed, this patient may have been typed as Rh negative by other testing centers.*

b) ***Neonatal specimens****: Sample demonstrates weak expression of the D antigen. For the purpose of maternal RhIg eligibility, this neonate should be considered RhD positive and the RhD negative birth parent a candidate for RhIg prophylaxis. For the purposes of transfusion, this neonate should be considered RhD negative.*

c) ***Cord specimens****: Sample demonstrates weak expression of the D antigen. For the purpose of maternal RhIg eligibility, this cord sample should be considered RhD positive and the RhD negative birth parent a candidate for RhIg prophylaxis.*

When weak D (RhIAT) testing cannot be performed due to a positive Direct Antiglobulin Test or recent red blood cell transfusion the appropriate comment below is to be attached to the result.

a) *Unable to report weak D test due to positive DAT. For the purposes of maternal RhIg eligibility, the RhD negative birth parent is a candidate for RhIg prophylaxis.*

b) *Unable to perform weak D test due to recent transfusion of red blood cells. For the purposes of maternal RhIg eligibility, the RhD negative birth parent is a candidate for RhIg prophylaxis.*

In an effort to avoid duplication of testing, genotyping results should be shared with provincial reference laboratories. When applicable, referring hospitals are responsible to forward genotyping results to the QEII and/or IWK. All genotyping requests for prenatal and patients under the age of 16 should be forwarded to the IWK Transfusion Services. When prenatal genotyping is requested from outside the IWK the referral hospital should confirm with the IWK Transfusion Services that previous testing is not already on file. In the event genotyping is requested on hemoglobinopathy patients or chronically transfused patients 16 years of age and over the referring hospital should confirm with the QEII Transfusion Services if previous testing is already on file or if they require a copy of the results once they are received.

6. Important Information for Clinicians

Nova Scotia implemented its' standardized approach for Rh testing in 2015. Before this the province lacked a standard practice in testing and interpreting Rh typing. It is imperative that clinicians recognize that, based on the standardized testing method, patients who were tested before 2015 may have their RhD type changed from their historical RhD type. An algorithm for physicians has been developed in collaboration with the Rh Program of Nova Scotia to simplify the way in which Rh Indeterminate patients should be treated with regards to RhIg (Appendix C). All indeterminate results will be reported to the Rh Program of Nova Scotia, who will notify the primary caregivers. Final results and recommendations will also be forwarded when they become available.

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Appendix A: Commonly used Commercially Available D Antisera in Canada

Reagent	Anti-D		Specificity
	IgM	IgG	
Ortho (ID-MTS)	MS201	NA	Limited
Immucor Series 4	MS201	MS26	Limited
Immucor Series 5	TH28	MS26	Limited
Seraclone Anti-D Blend	BS232	BS221 & H41 11B7	Broad
NovaClone	D175-2	D415 1E4	Broad
Ortho Bioclone	MAD-2	Human Polyclonal	Broad

Appendix B: RhD Typing Flowchart

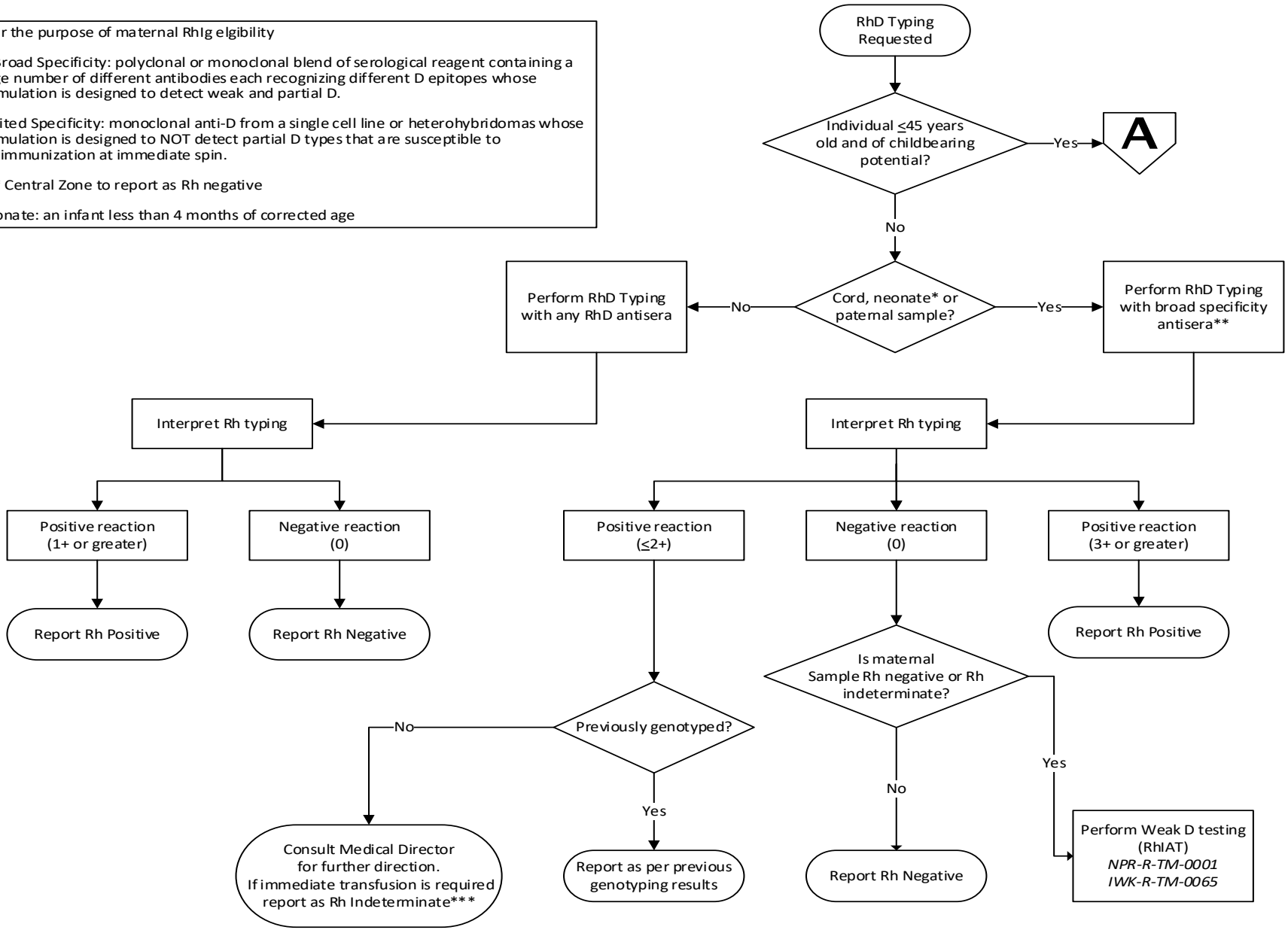
*For the purpose of maternal RhIg elgibility

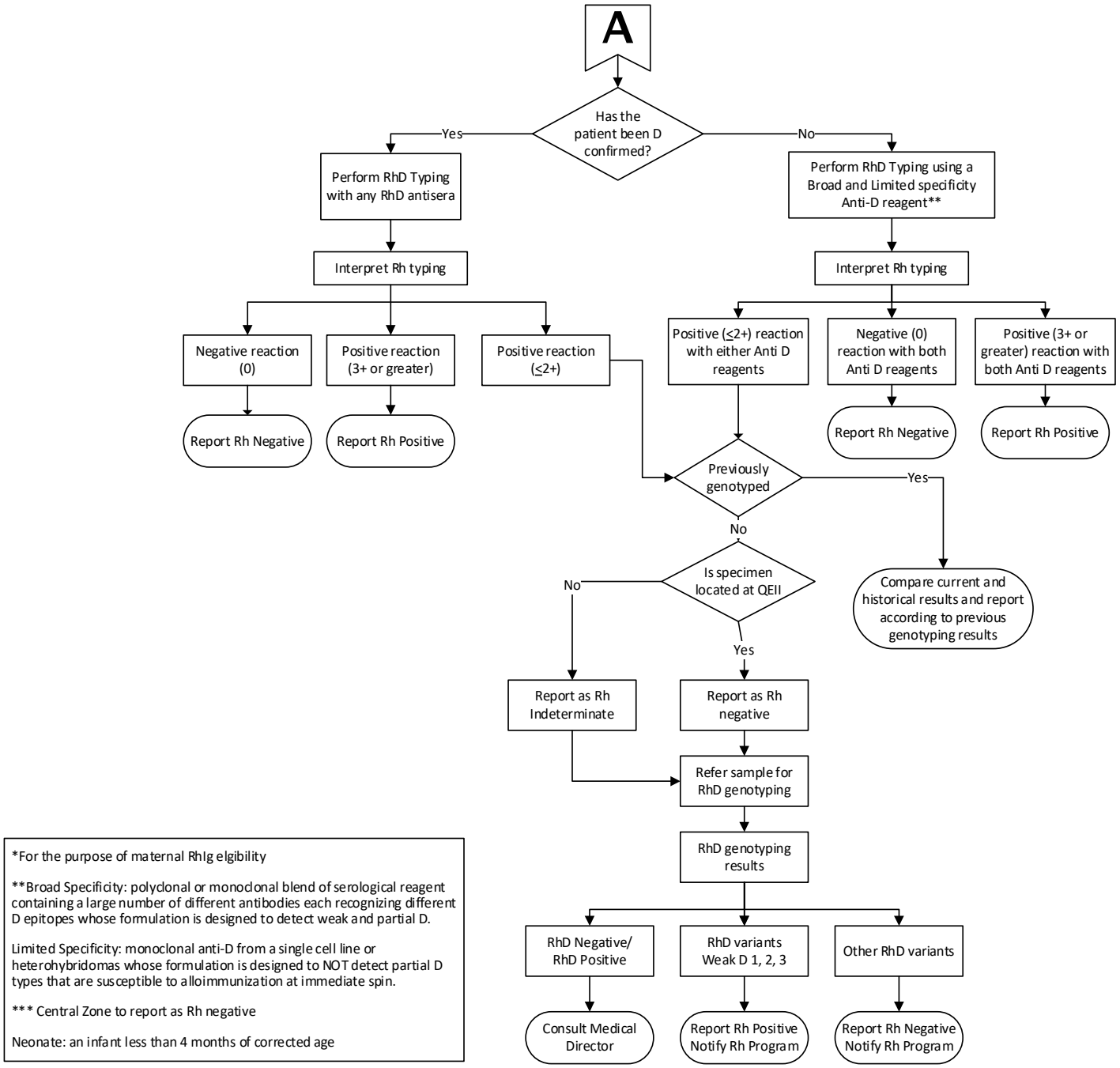
**Broad Specificity: polyclonal or monoclonal blend of serological reagent containing a large number of different antibodies each recognizing different D epitopes whose formulation is designed to detect weak and partial D.

Limited Specificity: monoclonal anti-D from a single cell line or heterohybridomas whose formulation is designed to NOT detect partial D types that are susceptible to alloimmunization at immediate spin.

*** Central Zone to report as Rh negative

Neonate: an infant less than 4 months of corrected age





*For the purpose of maternal RhIg elgibility

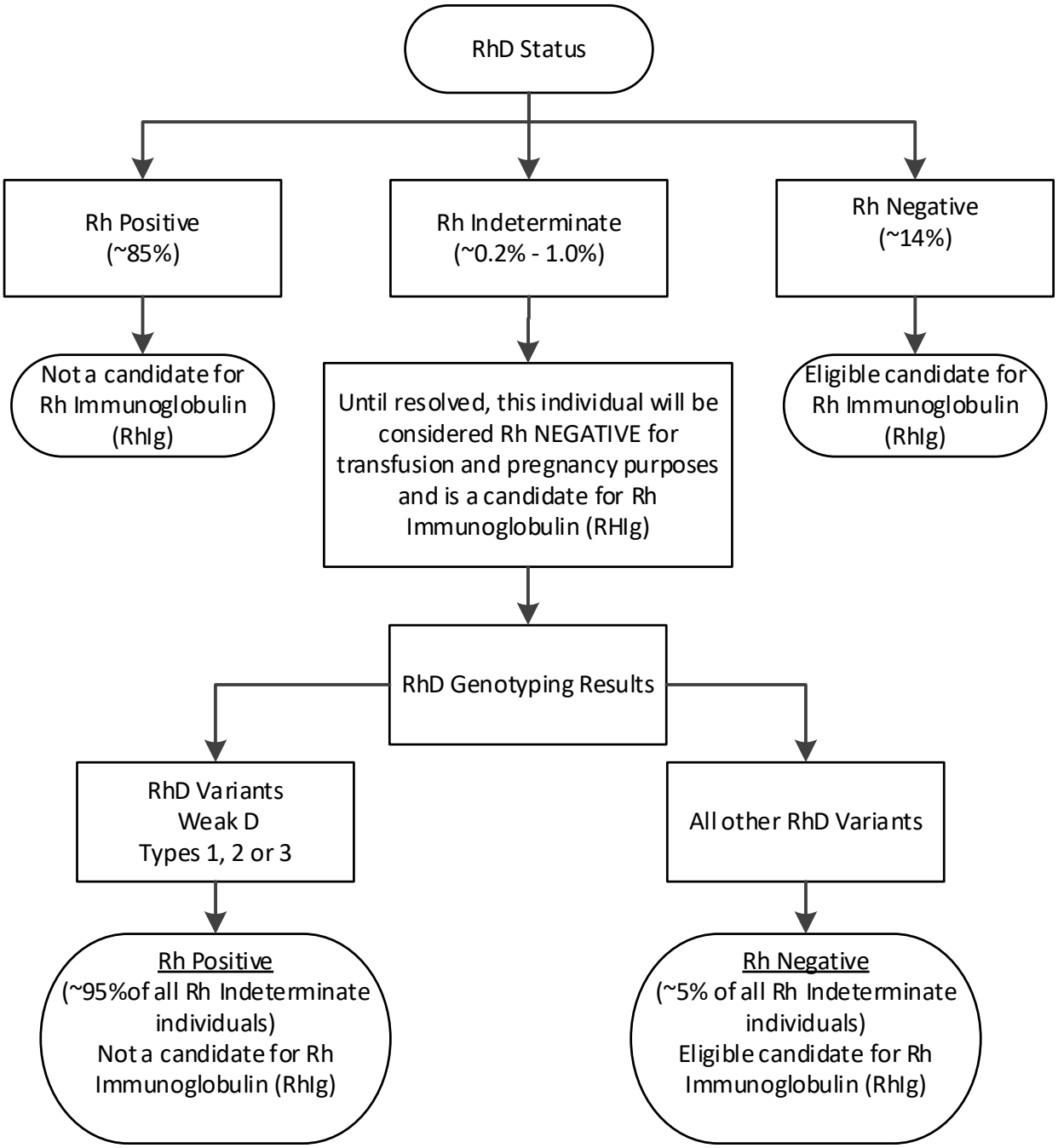
**Broad Specificity: polyclonal or monoclonal blend of serological reagent containing a large number of different antibodies each recognizing different D epitopes whose formulation is designed to detect weak and partial D.

Limited Specificity: monoclonal anti-D from a single cell line or heterohybridomas whose formulation is designed to NOT detect partial D types that are susceptible to alloimmunization at immediate spin.

*** Central Zone to report as Rh negative

Neonate: an infant less than 4 months of corrected age

Appendix C: RhD Interpretation Algorithm for Physicians



Appendix D: Interpretation

- ### RhD Confirmatory Two D Antisera Typing

Anti-D (Reagent 1) Limited specificity	Anti-D (Reagent 2) Broad Specificity	Interpretation
> 2+	> 2+	RhD Positive
0	0	RhD Negative
≤ 2+	≤ 2+	RhD Indeterminate Genotyping to be performed in select patients*
≤ 2+	> 2+	RhD Indeterminate Genotyping to be performed in select patients*
> 2+	≤ 2+	RhD Indeterminate Genotyping to be performed in select patients*

* Prenatal specimens are to be sent for genotyping, referral for all other specimen types will be determined on a case by case bases by the Transfusion Medical Director or designate.

- ### RhD Broad Specificity Single D Antisera Typing

Anti-D (Broad Specificity)	RhD Interpretation
> 2+	RhD Positive
0	RhD Negative*
≤ 2+	RhD Indeterminate** Genotyping to be performed in select patients

*If Cord, Neonate or Paternal sample is being tested for maternal RhIg eligibility perform Weak D (RhIAT) testing.
** If Cord, Neonate or Paternal sample is being tested for maternal RhIg eligibility consult Transfusion Medical Director for interpretation.

- ### Weak D (RhIAT) Testing

Anti-D (Broad Specificity) IAT	DAT	Weak D Interpretation	RhD Interpretation
≥ 1+	Negative	Weak D Positive	RhD Indeterminate
≥ 1+	Positive*	Weak D Invalid	RhD Negative **
0	N/A	Weak D Negative	RhD Negative

*If Weak D (RhIAT) positive a DAT must be performed. Positive weak D (RhIAT) test results are valid only if it can be shown that the red cells exhibit a negative DAT.
** With addition of comment**: "***Unable to report weak D test due to positive DAT. For the purposes of maternal RhIg eligibility, the RhD negative birth parent is a candidate for RhIg prophylaxis."***

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