Beaumont Laboratory
Royal Oak

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Related Documents: P061, P305, P502, P506, P512, P623F, ~~P716, P717, P718~~ ORTHO VISION Analyzer QC, Routine Testing on the ORTHO VISION Analyzer, ORTHO VISION Analyzer Manual Card Review

Rh(D) TYPING OF NEONATAL SAMPLES TO ASSESS MATERNAL RhIG CANDIDACY

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Purpose

The purpose of this document is to provide policies and procedures that apply when Rh(D) testing neonatal samples to assess maternal Rh Immune Globulin (RhIG) candidacy.

Scope

This document applies only to the Rh(D) testing of neonatal samples. The neonatal Rh(D) results are used to assess maternal RhIG candidacy as described in P502, *Rh Immune Globulin.*

Policies

The preferred method of Rh(D) testing of neonatal samples to assess maternal RhIG candidacy is in gel on the ORTHO VISION™. The alternate method of Rh(D) testing of neonatal samples is manually in tube.

The preferred sample for Rh(D) testing to assess maternal RhIG candidacy is a cord blood sample collected at the time of birth. The alternate sample for Rh(D) testing to assess maternal RhIG candidacy is a heel stick in a microtainer.

As with all manual tests, batch testing must be limited to 6 tests per batch. If workload becomes excessive, supervisory staff must be notified immediately.

Cord Blood Labeling Policies / Not Acceptable for Transfusion Purposes

- Refer to Transfusion Medicine policy ~~P101~~, *Triaging and Identifying Acceptable Samples for Testing* for the acceptable criteria of cord blood samples.
- Cord blood samples are not used for transfusion purposes. If a cord blood sample is received with the wristband number, then the wristband number is removed from the Band Number field of the Blood Bank computer system. Refer to the Triage CDM flow *Triaging Cord Blood Samples*.
- Cord blood samples from multiple births should not be collected, and if they are collected and sent to the Blood Bank they should not be tested for any purpose. Heel stick samples must be collected for testing from multiple births.

Heel Stick Samples / Labeling Policies

In the event that a cord blood was not tested for RhIG purposes, a heel stick must be tested to determine RhIG candidacy. If a heel stick is received for a Rh for Rhig - Newborn

(NBRHR) order, a Newborn Type and DAT (NBTD) must be added on to the sample, and the NBRHR will be canceled. For additional information, refer to the policy *Appropriate Blood Bank Computer Test Codes, Anti-D Reagent, and Control*.

Heel stick samples must be labeled as described in Transfusion Medicine policy ~~P101~~, *Triaging and Identifying Acceptable Samples for Testing.* Heel stick samples are labeled with a wristband number and may be used for transfusion purposes. Refer to the Triage CDM flow *Triaging Neonatal Heel Stick Samples.*

Washing Samples Before Manual Tube Testing

- All cord blood samples must be adequately washed before manual tube testing due to potential contamination with Wharton's jelly.
- If a heel stick sample shows visible hemolysis, lipemia, or icterus then the sample must be adequately washed before testing.

Neonatal Samples must be Collected for all Rh(D) Negative or Weak D Positive Mothers

- All Rh(D) negative or weak D positive mothers must be evaluated for RhIG candidacy after delivery; therefore a neonatal sample must be collected in these cases.
- If a cord blood sample is received on the neonate of a Rh positive mother, Rh(D) testing on the neonatal sample is not indicated.

Appropriate Blood Bank Computer Test Codes, Anti-D Reagent, and Control

ORTHO VISION™ Gel Testing: The MTS™ Anti-D Monoclonal IgM cards are used for neonatal Rh(D) testing for RhIG purposes on the ORTHO VISION™. The MTS™ Control cards must be tested in parallel with the MTS™ Anti-D Monoclonal IgM cards. The MTS™ Anti-D Monoclonal IgM cards are used for the following test:

- **NBRHR** (Newborn Rh for RhIG): This test is performed on all samples (for neonatal Rh(D) testing for RhIG purposes). The results of this test will interface to the HIS.

  Note: If the neonatal Rh(D) testing for RhIG purposes is performed in gel on the ORTHO VISION™, a repeat Rh(D) test is not required even if the neonate is Rh(D) negative in the NBRHR test.

Manual Tube Testing on a cord blood: The appropriate Anti-D reagent for manual tube neonatal Rh(D) testing for RhIG purposes is the Gamma-clone Anti-D reagent. The Gamma-clone control must be tested with the Gamma-clone Anti-D reagent. The Gamma-clone Anti-D reagent is used for both of the following tests:

- **NBRHR** (Newborn Rh for RhIG): This test is performed on all samples (for neonatal Rh(D) testing for RhIG purposes). The results of this test will interface to the HIS.
- **RRHTT** (Repeat Rh Test Tube): This test is performed if the neonate is Rh(D) negative in the NBRHR test. The results for the RRHTT code do not interface to the HIS. The RRHTT must be performed by a second / different technologist than the technologist who performed the NBRHR.

Manual Tube Testing on a heel stick: When a NBDT is ordered for a neonate and the Rh(D) testing is for RhIG purposes, the appropriate Anti-D reagent for a heel stick is determined based on the test requested. The reagent tested for the immediate spin reaction will be the

one carried through weak D if the neonate is Rh(D) negative, and the test is for RhIG purposes.

- **NNPR** (No Previous Newborn): This test is performed when a neonate has had no previous type in the LIS. The correct Anti-D reagent for this test is the Ortho BioClone Anti-D. The Ortho BioClone Anti-D is tested in tandem with the 7% BSA if the Rh(D) is taken through weak D testing. The results of this test will not interface to the HIS.
- **NTYPE** (Newborn ABORh): This test is performed on all heel stick samples (except those requiring only an HDN survey. The appropriate Anti-D reagent for manual tube neonatal Rh(D) testing for RhIG purposes is the Gamma-clone Anti-D reagent. The Gamma-clone control must be tested with the Gamma-clone Anti-D reagent. The results for the NTYPE code do interface to the HIS. The NTYPE must be performed by a second / different technologist than the technologist who performed the NNPR.

Reactive MTS™ Control Card or Gamma-clone Control

A negative MTS™ Control card or Gamma-clone control is required for Rh(D) interpretation. If the MTS™ Control card is reactive, the Rh(D) testing should be repeated manually in tube using the Gamma-clone Anti-D reagent and Gamma-clone control. If the Gamma-clone control is reactive, then the RBCs should be washed several times in saline (warm saline may be used) before testing. If the control is still reactive after washing the RBCs in saline, then the Rh(D) cannot be interpreted; the RND interpretation will be made (Rh not determined). If applicable, refer to P623, Attachment F, *Resolution of Rh(D) Discrepancies*. Refer also to the *Quality Control* and *Interpretation* sections of this document.

Definitions

- **Neonate:** Patients less than 4 months old.
- **HDN:** Hemolytic Disease of the Newborn
- **Weak D:** An expression of the D antigen demonstrated after incubation and the addition of antiglobulin serum, formerly known as Du.
- **HIS:** Hospital Information System
- **DAT:** Direct Antiglobulin Test

Specimen Collection and Handling

- Cord blood samples drawn into properly labeled EDTA tubes are the routine specimen for this procedure.
- Properly labeled heel stick samples collected in microtainers or EDTA tubes may be used as well.
- EDTA anticoagulated patient samples should be centrifuged if testing is performed in gel on the ORTHO VISION™.
- For additional information refer to the policies *Cord Blood Labeling Policies / Not Acceptable for Transfusion Purposes* and *Heel stick Samples / Labeling Policies.*

Reagents

- Gamma-clone Anti-D
- Gamma-clone control

- Ortho BioClone Anti-D
- 7% BSA
- Monospecific Anti-IgG
- IgG coated check cells
- MTS™ Anti-D Monoclonal IgM cards
- MTS™ Control cards

Equipment / Supplies

- table top centrifuge
- lighted viewing mirror
- 10 x 75 mm or 12 x 75 mm test tubes
- ~~13 x 100 mm test tubes~~
- disposable pipettes
- normal saline
- Wooden applicator sticks
- ORTHO VISION™ Analyzer

Quality Control

- **ORTHO VISION™ Rh(D) Testing**
  - Daily quality control for the ORTHO VISION™ instruments are performed as described in Transfusion Medicine policy, ~~P716,~~ *ORTHO VISION™ Analyzer QC*.
  - A negative result in the MTS™ Control card is required for Rh(D) interpretation.

- **Manual Tube Rh(D) Testing on a Cord Blood**
  - Daily quality control of ABO and Rh testing is documented in the Blood Bank computer system; see the *BBCDM.* See also P305, *Routine Quality Control of Blood Bank Reagents.*
  - A negative Gamma-clone control is required for Rh(D) interpretation.
  - Weak D testing should not be performed on samples with a positive direct antiglobulin test (DAT); false-positive weak D results may be obtained. Note that DATs are not routinely performed. Therefore, the Gamma-clone control is tested in parallel with the weak D test. The Gamma-clone control must be negative at the antiglobulin phase in order to interpret the weak D test.
  - IgG-coated RBCs are used as a control if the graded reaction of the weak D test is negative at the AHG phase. The ~~graded~~ reaction after the addition of the IgG-coated RBCs must be positive ~~at least 2+.~~ If these requirements are not met, then the weak D test is not valid and must be repeated.

Procedure: Rh(D) Testing of the Neonate for RhIG Purposes Using the Gel Method on the ORTHO VISION™ Analyzer

Note: Cord blood samples drawn in EDTA tubes are required when performing neonate Rh(D) testing on the ORTHO VISION™. If a heel stick is drawn in a microtainer, the Rh(D) testing must be performed using the manual tube method.

1. Ensure that the NBRHR test is ordered under the accession number corresponding to the patient sample.

2. Rim out the patient sample using wooden applicator sticks to remove any clots that may be present.

3. Centrifuge the patient sample for 10 minutes to obtain packed red blood cells.

4. Load the patient sample onto the ORTHO VISION™. Testing should begin automatically once the samples are scanned by the instrument.
   - Specimen caps should be removed prior to loading on the ORTHO VISION™.
   - Refer to Transfusion Medicine policy, ~~P717,~~ *Routine Testing on the ORTHO VISION™ Analyzer* for additional information.
   - If the ORTHO VISION™ is unable to perform the Rh(D) testing, testing should be performed using the manual tube method.

5. Upon completion, the test results will either interface to the Blood Bank computer system automatically or require manual review.
   a. If the test results interface automatically, they should be verified in the Blood Bank computer system as described in Triage CDM / *Completing Vision™ Results*.
   b. If the test results require manual review, proceed as described in Transfusion Medicine policy, ~~P718,~~ *ORTHO VISION™ Analyzer Manual Card Review*.

Procedure: Rh(D) Testing of the Neonate for RhIG Purposes Using the Manual Tube Method on a Cord Blood

1. Label a 12 x 75 ~~13 x 100~~ mm test tube to identify the neonatal cell suspension.

2. Label two 10 x 75 mm or 12 x 75 mm test tubes for the following:
   - Gamma-clone Anti-D and neonatal RBCs
   - Gamma-clone control and neonatal RBCs

3. In the 12 x 75 ~~13 x 100~~ mm test tube, prepare a 3-4% suspension of the neonate's RBCs.
   - If using a cord blood sample, wash the RBCs 1 time with saline; then resuspend to 3-4%. If the Gamma-clone control is positive, wash the RBCs additional times.
   - If a heel stick sample shows visible hemolysis, lipemia, or icterus then wash the RBCs 3 times with saline; then resuspend to 3-4%.

4. Place 1 drop of the Gamma-clone Anti-D reagent into the correspondingly labeled test tube.

5. Place 1 drop of the Gamma-clone control into the correspondingly labeled test tube.

6. Add 1 drop of the neonate's 3-4% RBC suspension to each of the following tubes . . .
   - The tube containing the 1 drop of Anti-D reagent.
   - The tube containing the 1 drop of control.

7. Mix thoroughly by shaking and centrifuge the tubes according to calibrated time.

Note that it is not necessary to centrifuge immediately; centrifugation may be delayed up to 30 minutes while the tubes remain at room temperature (23°C ± 3°C).

8. Examine the tubes for hemolysis. Then gently resuspend the RBCs by gently shaking. Read and grade the reactions macroscopically.
   - Note that hemolysis may be the consequence of bacterial contamination and should not be interpreted as a positive result.
   - Refer to P061, *Reading, Grading, and Recording Test Reactions*.

9. Record the graded reactions in the Blood Bank computer, using the NBRHR test code.
   Refer to the CDM flow *Resulting Neonatal Testing under Patients / Orders / Results* or to the flow *Resulting Neonatal Testing on a Worksheet*.

10. Evaluate the graded reactions of the tube with the Anti-D reagent.
    - If the graded reaction of the tube with the Anti-D reagent is negative, weak+, or 1+ then perform the weak D test as described in steps 11- 16.
    - If the graded reaction of the tube with the Anti-D reagent is positive (2+, 3+, or 4+) then proceed to step 16.

11. Incubate the 2 tubes (containing the anti-D reagent and the control) for 15 minutes at 37°C ± 1°C.
    Incubation may be extended for up to 30 minutes, if desired. Incubation for the upper end of this time range may enhance reactivity.

12. Wash the tubes at least three times in tubes filled with saline, decanting the saline completely after the last wash.
    The washing phase of the weak D test must be carried out without interruption, and the reactions should be graded immediately after addition of the anti-IgG reagent.

13. To each of the two tubes, add two drops of monospecific Anti-IgG.

14. Mix thoroughly by shaking and centrifuge the tubes immediately, according to calibrated time.

15. Resuspend the RBCs by gently shaking. Read and grade the reactions of the weak D test macroscopically.

16. Record the graded reactions of the weak D test in the Blood Bank computer, using the NBRHR test code.
    Refer to the CDM flow *Resulting Neonatal Testing under Patients / Orders / Results* or to the flow *Resulting Neonatal Testing on a Worksheet*.

17. Add IgG coated cells to the test tube(s) if the graded reaction is negative at the AHG phase. Agitate tubes to mix. Centrifuge according to calibrated time.
    The graded reaction after the addition of the IgG-coated RBCs must be positive (any strength) ~~at least 2+.~~ If these requirements are not met, then the weak D test is not valid and must be repeated.

18. Interpret the results as described in the *Interpretation* section. Record the interpretation in the Blood Bank computer. If the NBRHR is interpreted as Rh(D) negative, this procedure should be repeated by a second / different technologist and resulted under a RRHTT test code.

Interpretation

Interpretation of Neonatal Rh(D) Type for Maternal RhIG Candidacy (ORTHO VISION™ Gel)

	MTS™ Anti-D Monoclonal IgM cards	MTS™ Control card	**Rh Interpretation**
Valid Interpretation	4+	0	Rh(D) positive
	0	0	Rh(D) negative
	w+, 1+, 2+, or 3+	0	Weak D
	~~3+~~	~~0~~	~~* See note below~~
Invalid Interpretation	If the MTS™ Control card is reactive (any strength), the Rh(D) testing should be repeated manually in tube using the Gamma-clone Anti-D reagent and Gamma-clone control. Refer to the *Reactive MTS™ Control Card or Gamma-Clone Control* section for additional information.		

~~*** Note:** All 3+ gel Rh(D) reactions must be tested in tube before interpretation. If the tube Rh(D) reaction is 2+, 3+, or 4+, the patient will be interpreted as Rh(D) Positive. If the tube Rh(D) reaction is 0, w+, or 1+, the patient will be interpreted as weak D positive due to the discrepancy.~~

Interpretation of Neonatal Rh(D) Type for Maternal RhIG Candidacy (Tube Method)

- **D:** Reaction with Anti-D
- **DC**: Reaction with D control
- **Du:** Reaction with Anti-D at the antiglobulin phase of the weak D test
- **DuC:** Reaction with D control at the antiglobulin phase of the weak D test
- **Ducc:** Reaction of the check cells (in the tube with the anti-D reagent) in the weak D test
- **DuCcc**: Reaction of the check cells (in the tube with the control reagent) in weak D test
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*	D	DC	Du	DuC	Ducc	DuCcc	Rh Interpretation
Valid Interpretation	2+ or greater	0	NT	NT	NT	NT	Rh(D) positive
	0	0	0	0	positive (any strength) ~~2+ or greater~~	positive (any strength) ~~2+ or greater~~	Rh(D) negative
			w+, 1+, 2+, 3+, 4+	0	NT	positive (any strength)	Weak D

						2+ or greater	
	w+ or 1+	0	NT 0	NT0	NT 2+ or greater	NT 2+ or greater	
			NT w+, 1+, 2+, 3+, 4+	NT 0	NT	NT 2+ or greater	
Invalid Interpretation	• If the Gamma-clone control is reactive (any strength / at any phase that was tested) then the RBCs should be washed several times in saline (warm saline may be used) before testing. If the control is still reactive after washing the RBCs in saline, then a valid Rh(D) interpretation cannot be made. • If check cells are required (because the graded reaction of the weak D test is negative at the AHG phase), then the check cells must react positive (any strength) at least 2+. If the check cells do not react positive (any strength) at least 2+. then the weak D test is not valid and must be repeated. If, after repeating, the check cells still do not react positive (any strength) at least 2+. then a valid Rh(D) interpretation cannot be made. • For additional information, refer to P623, Attachment F, *Resolution of Rh(D) Discrepancies.*						RND (Rh not determined)

*****The column headers in the table below correspond to the fields of the NBRHR and RRHTT tests in the Blood Bank computer.

References

- AABB, *Technical Manual*, current edition.
- Immucor/Gamma *Anti-D (Monoclonal Blend) Gamma-clone*® IFU, 10/2007.
- Immucor/Gamma *Gamma-clone*® *Control* Instructions for Use, revised 10/2007.

Authorized Reviewers

Chief, Pathology and Laboratory Medicine
Medical Director and/or Designee, Blood Bank
Manager/Supervisor, Blood Bank

Rh(D) TYPING OF NEONATAL SAMPLES TO ASSESS MATERNAL RhIG CANDIDACY

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