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| **Rh Typing – Tube Testing**  |
| **Purpose** | This procedure provides instruction for the testing of patient red cells for the presence of the Rh (D) antigen, including testing for weak D when required.  |
| **Policy Statements** | * Seraclone Control ABO+Rh must be run on samples testing positive with Anti-A, Anti-B and Ant-D on the patient’s first typing and as part of the first typing ABO/Rh recheck.
* ABO/RH Rechecks on specimens drawn by electronic identification verification system, <4 months and/or type as group O:
* *Group O, Rh negative red cells and group AB platelets or plasma shall be selected for transfusion until confirmation of the patient’s ABO has been completed by a second technologist.*
1. Confirmatory testing may be performed using the same sample but with a new cell

 suspension.1. An Rh control shall be performed as part of the ABO recheck on new AB, Rh Positive

 patients.1. Weak D testing does NOT need to be performed as part of the ABO/Rh recheck

 unless the patient’s red cells tested positive at AHG on initial testing.1. Both technologists shall perform discrepancies resolution testing.
* ABO/RH Rechecks on patients not drawn by an electronic identification verification system and are >4 months and type as non-O.
	1. A second independent sample needs to be tested to confirm blood type. Either a previous sample can be used or a new specimen needs drawn for an ABO/Rh.
	2. Follow the policy statements above for patients that are collected by an electronic identification system.
	+ Weak D testing will be performed on the following:
	1. Infants ≤7 days old whose red cells show a negative reaction with Anti-D reagent at

 immediate spin. *Infants < 4 months of age that test negative with anti D at immediate spin shall* *receive Rh negative red cells regardless of the infants weak D testing results.** 1. Potential direct donors whose red cells show a negative with Anti-D reagent at

 immediate spin.* 1. Any patient whose red cells show a w+ reaction with Anti-D reagent at immediate

 spin. * Students may only perform ABO/Rh testing on patients with a minimum of two ABO/Rh tests on record.
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| **Test Codes** | [ABO/Rh](http://www.childrensmn.org/Manuals/Lab/TransfusionSvc/012704.asp) [Rh Only](http://www.childrensmn.org/Manuals/Lab/TransfusionSvc/012738.asp).As part of other testing battery (Type and Screen, Newborn Workup, etc)ABR-ABO and Rh ARC-ABO and Rh Recheck |
| **Related****Documents** | TS 4.1 Making a 3-5% Cell SuspensionTS 4.8 Grading and Interpretation of Tube ReactionsTS 4.4 Cell Washing-Manual MethodTS 4.13 Direct Antiglobulin Test, Anti-IgG TubeTS 4.36 Resolving a D Typing Discrepancy |
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| **Materials** | **Equipment** | **Reagents** | **Supplies** |
| * Centrifuge
* Agglutination Viewer
 | * BioRad Anti-D (Rh1) Blend
* Seroclone Control ABO+Rh
* BioRad Coombscell-E
 | * 10 x 75 mm test tubes
* BB pipettes
* Saline
* Marker
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| **Sample** | Fresh patient samples of EDTA or clotted whole blood collected following general blood collection procedures are acceptable. See [Collection of Patient Specimens](http://www.childrensmn.org/Manuals/Lab/TransfusionSvc/012709.asp).Citrated samples from donor unit segments or pilot tubes.EDTA or citrated anti-coagulated whole blood samples must be used for weak D testing.The specimen should be tested as soon as possible after collection. If testing is delayed, the EDTA or clotted specimen should be stored at 2-6°C and may be tested within 10 days from collection. Donor blood may be tested until the products expiration date. Specimens exhibiting gross hemolysis or contamination should not be used. |
| **Quality Control** | Refer to TS 18.2 Performing Daily Reagent Quality ControlReagents must be evaluated each day of use with appropriate controls. Control for Weak D testing:Anti-IgG AHG tube reagent and Coombs Control Cells day of use QC. |
| **Before** **You Begin** | 1. Confirm sample acceptability and review patient history per procedure.
2. Label tubes per TS 4.6 Labeling Tubes.
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| **Procedure** |  |
|  | **Step** | Action |
|  | 1 | Prepare a 3-5% cell suspension of patient red cells in isotonic saline. |
|  | 2 | Add 1 drop Anti-D antiserum AND 1 drop patient 3-5% red cell suspension to the labeled tube and mix. |
|  | 3 | Incubate tubes at room temperature for 5 to 10 minutes |
|  | 4 | Centrifuge for the posted optimal time in a calibrated serologic centrifuge. |
|  | 5 | Remove the tubes from the centrifuge. |
|  | 6 | Gently resuspend the cell button and examine macroscopically for hemolysis and agglutination immediately after centrifuging. |
|  | 7 | Immediately record the graded reactions in the computer or on the downtime worksheet. |
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| **If the reaction at immediate spin with Anti-D is** | **Then**  |
| 1+ or greater | Testing is completed and all other phases may be resulted as NT. Interpret the results as Rh Positive. |
| w+ | Perform Weak D testing |
| 0 and patient is ≤ 7 days | Perform Weak D testing |
| 0 and patient is > 7 days | Testing is completed and all other test phases may be resulted as NT. Interpret the results as Rh Negative. |

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| **\*New AB, Rh Positive patients**  **or****\*RH Only test**  | 9 | Test the patient's red cell suspension with a Seraclone Control ABO+Rh* Place 1 drop of the control reagent into new tube labelled as RHC.
* Add 1 drop of the patient's 3% cell suspension to the RHC tube
* Spin the tube in centrifuge for the time indicated.
* Gently resuspend cells completely and examine for agglutination.
* Record the results in the computer or on the downtime worksheet

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| **If the RHC reaction is** | **Then ABO/Rh result is** |
| Negative | Valid |
| Positive | Invalid-Forward to Reference lab to determine patient's ABO/Rh. |

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|  | 10 | Compare the current D (Rh) results with any previous results.

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| **If a previous record** | **And the current and previous results**  | **Then** |
| Exists | Agree | Finalize the results in the computer or on the worksheet  |
| Do not agree | Resolve the discrepancy. |
| Does not exist and patient is collected by electronic identification verification system, <4 months of age and/or types as O | N/A | Finalize results. Add test ARC (ABO /Rh recheck) to the order if needed and forward sample for a second ABO/Rh by a second technologist |
| Does not exist and is not collected by electronic identification verification, >4 months of age, and/or types as Non group O | N/A | * Order an ABO/Rh (ABRH) as no charge.
* Check to see if a previous sample was drawn to perform testing. (e.g. CBC)
* If no previous sample, call patient’s nurse and ask if they want lab to draw new specimen or are they going to draw specimen.
* Perform ABO/Rh and have second tech perform ARC
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|  | 11 | Review the final record including a final clerical check of sample, label, request, and interpretation. |
|  | 12 | Dispose of all tubes and pipettes used for the examinations in a biohazard waste container. |
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|  | **Step** | Action |
| **Weak D Testing** | Continuing from step 7above. |
| 1 | Incubate tube (patient red cells and Anti-D) for 15-30 minutes at 36-38 °C. |
| 2 | Centrifuge for posted time. |
| 3 | Gently resuspend the cell button and examine macroscopically for hemolysis and agglutination immediately after centrifuging. |
|  | 4 | Immediately record the results in the computer or on the downtime worksheet.

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| **If the reaction after 37 °C is** | **Then** |
| ≥1+ agglutination | Testing is completed and all other phases may be resulted as NT. Interpret the results as Rh Positive.  |
| No agglutination or w+ agglutination | Proceed to step 5. |

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|  | 5 | Wash the tube 4 times with isotonic saline decanting completely after the final wash. |
|  | 6 | Add 2 drops Anti-IgG AHG to the tube and mix gently. |
|  | 7 | Centrifuge for the time indicated. |
|  | 8 | Gently resuspend the cell button and examine macroscopically for hemolysis and agglutination per established procedure. |
|  | 9 | Immediately record the graded reactions in the computer or on the downtime worksheet. |
|  | 10 | Validate all negative antiglobulin results:1. Add 1 drop of Coombscell-E to the negative tube.
2. Centrifuge for the posted time in a calibrated serologic centrifuge.
3. Resuspend the cells.
4. Read macroscopically for agglutination and record the results.

Valid control results: Agglutination with Coombscell-E must be present or the AHG test results are invalid and the test must be repeated. |
|  | 11 | If agglutination is present at AHG perform a DAT using patient’s washed cells from the same specimen.Note: St. Paul **DO NOT** interpret weak D testing until DAT is resulted.

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| **If the DAT is** | **Then** |
| Positive | No valid interpretation can be made. Interpret Rh as Inconclusive.* Transfuse with Rh negative cells as needed.
* Forward to reference lab for Rh determination
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| Negative | Interpret as D (Rh) Positive. Add Problem Patient comment WDP to patient’s BAD file. |

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|  | 12 | Compare the interpretation with the historical patient record.

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| **If the historical and current results** | **Then** |
| Agree | Record the interpretation in the computer. |
| Disagree | Resolve the discrepancy. TS 4.36 Resolving a D Typing Discrepancy |

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|  | 13 | Review the computer record or worksheet result entry including a final clerical check of sample, label, and request. |
|  | 14 | Dispose of all tubes and pipettes used for the examination in a biohazard waste container. |
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| **Interpretation** |  |
| Anti-D and patient red cells | **Interpretation** |
| **IS** | **@ 37** | **@ AHG** | **CC** | **RHC** | **DAT-weak D only** |
| + | NT | NT | NT | NT or 0 | NT | Positive |
| 0 | + | NT | NT | NT or 0 | NT | Positive |
| 0 | 0 | + | NT | NT or 0 | Neg | Positive |
| 0 | w+ | + | NT | NT or 0 | Neg | Positive  |
| 0 | NT | NT | NT | NT | NT | Negative |
| 0 | 0 | 0 | + | NT  | NT | Negative |
| 0 | 0 | 0 | 0 | NT | NT | Invalid Test, repeat testing |
| 0 | 0 | + | NT | NT | Pos | Inconclusive |
|  |  |  |  | + |  | Invalid Test, repeat or sendout |
| + = 1+ or greater agglutination 0 = no agglutination NT=Not Tested IS=Immediate Spin@37C= after incubation at 36-38C@AHG=after addition of Anti-IgGDAT = Direct Antiglobulin Test (required only if positive reaction at AHG)RHC= Rh Control |
| **Limitations** | Tubes should be read immediately following centrifugation and delays may cause a dissociation of antigen-antibody complexes resulting in false negative or weak positive reactions.Cold agglutinins, a positive direct antiglobulin test, or rouleaux may cause false positive reactions. |
| **Result Reporting** | TS 5.6 Entering Results for ABO/Rh testing or for ABO/Rh RecheckTS 5.8 Entering Results for Rh Typing Only |
| **References** | *Product Insert, Blood Grouping Reagent, Anti-D (RH1) Blend, Bio-*Rad Medical Diagnostics, current edition*Product Insert, Blood Grouping Reagent, Seraclone Control ABO+RH, Bio-*Rad Medical Diagnostics, current edition *Product Insert, Anti-Human Globulin Anti-IgG, Bio*-Rad Medical Diagnostics, current edition*Product Insert, Coombscell-E, Bio*-Rad Medical Diagnostics, current edition |
| **Approval****Workflow** | Transfusion Service/Laboratory Director |
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | K. Hartley | 1983 | Initial Version |
| 2 | C. Berglund | 1985 |  |
| 3 | Wenzel/ McGee | 1990 |  |
| 4 | D. Hansen | 1995 |  |
| 5 | Hansen/Wenzel | 1996 | Merger |
| 6 | J. Wenzel | 9/1999 |  |
| 7 | J. Wenzel | 5/22/2001 |  |
|  | 8 | J. Wenzel | 6/05/2003 |  |
| 9 | Cassidy/Wenzel | 3/01/2008 | New format, update recheck testing policy |
| 10 | N. Poupard | 5/12/09 | Add Rh control if RH only ordered |
| 11 | J. Wenzel | 8/22/2011 | BioRad tube reagentsMerged with TS 4.18 Weak DEDTA or citrate samples only for weak D.Weak D testing if w+ reaction at IS Weak D testing as part recheck if positive at AHG for any patient tested, not just if patient < 7 days.Specify use of tube Anti-IgG AHG for weak D testing and subsequent DAT.Added LimitationsChange from 14 to 10 days specimen requirement. |
|  | 12 | J Wenzel | 4/10/2012 | Expand Interpretation table to match BMA4.4Removed requirement to perform a DAT using tube reagent as control in step 11 of weak D testing. |
|  | 13 | S. Cassidy | 11/18/15 | Added policy statement around second confirmatory testing of ABO/Rh |
|  | 14 | S. Cassidy | 11/13/2019 | Added a note that St. Paul will not report weak D testing until DAT has been performed |
|  | 15 | S. Cassidy | 07/22/2022 | Updated policy statement regarding two campuses |
|  | 16 | S. Cassidy | 07/01/2024 | Added room temp incubation per new package insert. |