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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. | | | | | | | | | | | | |
| **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 Suspension  TS 4.8 Grading and Interpretation of Tube Reactions  TS 4.4 Cell Washing-Manual Method  TS 4.13 Direct Antiglobulin Test, Anti-IgG Tube  TS 4.36 Resolving a D Typing Discrepancy | | | | | | | | | | | | |
|  |  | | | | | | | | | | | | |
| **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 | |
|  | | | | | | | | | | | | |
| **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 Control  Reagents 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. | | | | | | | | | | | | |
| **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. | | | | | | | | | | |
|  | 8 | | |  |  | | --- | --- | | **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. | | | | | | | | | | | |
| **\*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  |  |  | | --- | --- | | **If the RHC reaction is** | **Then ABO/Rh result is** | | Negative | Valid | | Positive | Invalid-Forward to Reference lab to  determine patient's ABO/Rh. | | | | | | | | | | | |
|  | 10 | | Compare the current D (Rh) results with any previous results.   |  |  |  | | --- | --- | --- | | **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 | | | | | | | | | | | |
|  | 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. | | | | | | | | | | |
|  |  | | | | | | | | | | | | |
|  | **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.   |  |  | | --- | --- | | **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. | | | | | | | | | | | |
|  | 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.   |  |  | | --- | --- | | **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 | | Negative | Interpret as D (Rh) Positive. Add Problem Patient comment WDP to patient’s BAD file. | | | | | | | | | | | |
|  | 12 | | Compare the interpretation with the historical patient record.   |  |  | | --- | --- | | **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 | | | | | | | | | | | |
|  | 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. | | | | | | | | | | |
|  |  | | | | | | | | | | | | |
| **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-IgG  DAT = 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 Recheck  TS 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 | | | | | | | | | | | | |
|  |  | | | | | | | | | | | | |
| **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 reagents  Merged with TS 4.18 Weak D  EDTA 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 Limitations  Change from 14 to 10 days specimen requirement. | | | |
|  | 12 | | | J Wenzel | | | | 4/10/2012 | | | Expand Interpretation table to match BMA4.4  Removed 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. | | | |