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| **Weak D-Manual Gel Method** |
| **Purpose** | This procedure provides instruction for the testing of patient red cells for testing for weak D. |
| **Policy Statements** | * 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.
* 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 4.13 Direct Antiglobulin Test, Anti-IgG TubeTS 4.36 Resolving a D Typing Discrepancy |
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| **Materials** | **Equipment** | **Reagents** | **Supplies** |
| * IH-Centrifuge L
* IH-Incubator L
* IH-Reader 24-Mpls
 | * IH-Anti-D Blend
* IH-LISS
* IH-Card AHG Anti-IgG
 | * 10 x 75 mm test tubes
* BB pipettes
* Saline
* Marker
* MLA 10 µL pipette
* MLA 50 µL pipette
* MLA 1000 µL pipette
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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 18.14 Performing Quality Control IH-Reader 24 or Manual SystemReagents 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.
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| **Procedure** |  |
|  | **Step** | Action |
|  | 1 | Allow reagents and samples to reach room temperature (18-25°) before use. |
|  | 2 | Prepare cell suspension by pipetting 1000 µL IH-LISS into an appropriately-labeled test tube. Add 10 µL patient packed red cells to the IH-LISS and mix. |
|  | 3 | Inspect the IH-Cards for evidence of damage to the foil or drying of the gel. Do not use if such evidence is observed. IH-Cards with splashes of gel in the reaction area may be centrifuged prior to use. |
|  | 4 | Label the IH-Card according to procedure TS 4.07 Inspection and Labeling of Gel Cards. |
|  | 5 | Carefully peel back the foil seal from the individual microtubes of the IH-Card being used for the testing, leaving the seal in place for microtubes not being used.Note: Once the foil has been removed from the microtubes, testing must be initiated as soon as possible (recommended within 20 minutes), to prevent drying of the microtubes. |
|  | 6 | Add 50 µL of the patient red cell suspension to the correspondingly labelled microtube on the IH-Card taking care to maintain the air gap between the cells in the upper reaction chamber and the AHG reagent in the column of the microtube. |
|  | 7 | Add 25 µL of IH-Anti-D Blend to the patient cell suspension in the microtube. |
| **\*** | 8 | Place the IH-Card in an IH-Card rack or the 24-card centrifuge head, place in the center card section of the IH-Incubator L and incubate for 15-20 minutes (with preset temperature of 37°±1°C). |
|  | 9 | Centrifuge the IH-Cards in the IH-Centrifuge L at the pre-programmed time and speed for the 24-card centrifuge head (10 minutes at 85 x G). |
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| If agglutination is present at AHG perform a DAT using patient’s washed cells from the same specimen.Note: St. Paul **DO NOT** interpret a positive 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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| 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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|  | 12 | Review the computer record or worksheet result entry including a final clerical check of sample, label, and request. |
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| **Interpretation** |  |
| Anti-D and patient red cells | **Interpretation** |
| **ABO/D Card** | **@ AHG** | **CLT/ RHC** | **DAT-weak D only** |
| + | NT | NT or 0 | NT | Positive |
| 0 | + | NT or 0 | Neg | Positive |
| 0 | + | NT or 0 | Neg | Positive  |
| 0 | 0 | NT  | NT | Negative |
| 0 | + | NT | Pos | Inconclusive |
|  |  | + |  | Invalid Test, repeat or sendout |
| + = 1+ or greater agglutination 0 = no agglutination NT=Not Tested @AHG=after addition of Anti-IgGDAT = Direct Antiglobulin Test (required only if positive reaction at AHG)CLT/RHC= Rh ControlAgglutination of the red blood cells as seen by clumps of cells caught within the gel column is a positive test and indicates the presence of the D antigen on the red cells. Grading of reaction follows procedure TS 4.09 Grading and Interpretation of Gel Card ReationsNo agglutination of the reagent red blood cells as observed by a smooth surfaced button at the bottom of the gel column is negative reaction and indicates that no unexpected antibody was detected.Following centrifugation cards should be read as soon as possible and results interpreted within 6 hours of processing. If unable to read within that time period, the IH-Cards can be sealed with tape and stored at 2-8°C for up to 24 hours. Further delays in reading and interpretations can lead to drying of the gel which interfere with accurate reading of the results. |
| **Limitations** | Erroneous and abnormal results may be caused by:* Bacterial or chemical contamination of the blood specimens, reagents, supplementary materials and/or equipment.
* Patient medication or disease yielding a cross-reaction.
* Incomplete re-suspension of the red blood cells.
* Sample or reagent red blood cell hemolysis prior to testing
* Contamination between microtubes through pipetting errors.
* Grossly icteric blood samples, blood samples with abnormally high concentrations of protein or blood samples form patients who have received plasma.
* Fibrin, clots, particulates or other artifacts may cause some red blood cells to be a trapped at the top of the gel and cause an anomalous result. They may appear as a pinkish layer. In a negative reaction, the false appearance of a mixed filed could lead to misinterpretation.
* If red blood cells (pellet at the bottom of the microtube) are too low in concentration, they become difficult to visualize and a weak positive reaction may fail to be detected.
* Red blood cells with positive Direct Antiglobulin test with Anti-IgG reagents may produce false positive results in the weak D test using the IH-Card Anti-IgG or Anti-IgG,-C3d.
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| **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: IH-Card AHG Anti-IgG Bio-Rad Medical Diagnostic Gmbh, current version**Blood Grouping Reagent, IH-Anti-D (RH1) Blend, Bio-*Rad Medical Diagnostics, current edition*Product Insert, IH-LISS Solution, 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 | S. Cassidy | 02/17/2023 | Initial Version |