|  |
| --- |
| **ABO Grouping– Gel Testing**  |
| **Purpose** | This procedure provides instructions for the gel testing of patient for A and B red cell antigens, and corresponding ABO group antibodies for the determination of ABO grouping. |
| **Policy Statements** | 1. **Patients > 4 months old:** ABO group shall be determined by testing the patient’s red cells with anti-A and anti-B reagents and by testing the patient’s plasma/serum for expected antibodies with A1 and B reagent cells.

 *If a discrepancy is found between the patient’s forward and reverse typing on patient’s*  *> 4 months old, additional testing shall be performed. Group O red cells must be selected* *for transfusion until the discrepancy is resolved or in the event the ABO typing is* *interpreted as inconclusive.* 1. **Patients < 4 months old:** ABO group shall be determined by testing the patient’s red cells with anti-A and anti-B reagent. Testing the patient’s plasma/serum for antibodies with A1 or B cells shall not be performed.
2. ABO/RH Rechecks on specimens drawn by electronic identification verification system, <4 months and/or type as group O:
3. *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 two different methods. If unable to perform two different methodologies then two techs have to perform the ABO/Rh.*
	1. Confirmatory testing may be performed using the same sample but using tube method.
	2. An Rh control shall be performed as part of the ABO recheck on new AB, Rh Positive patients.
	3. Resolution of ABO front and back typing discrepancies shall be performed as part of the recheck as needed.
	4. Reverse typing (A, and B cells) testing may be omitted and resulted as NT on the

recheck if QNS on patient plasma.1. 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
2. Any patient who is being evaluated for solid organ donation cannot have A1 subgroup typing performed if they have been transfused within the last three months.
3. Students may only perform ABO/Rh testing on patients with a minimum of two

 ABO/Rh tests on record.1. Double population (DP) resulting in the front type in gel cards
	1. ABO/Rh history on file
	2. Transfused non-type specific RBCs within 120 days from current testing that would cause double population
	3. Add comment “Patient transfused with XXX cells on XX/XX/XX” in the IH-Come resulting
	4. If there are any questions on the ABO/Rh typing or does not have ABO/Rh history or was not transfused with non-type specific RBCs with in the timeframe, reject gel card results and repeat in testing in tube.
2. A positive CLT in gel, the whole card needs to be rejected and repeated in tube.
 |
| **Test Codes** | [ABO/Rh](http://www.childrensmn.org/Manuals/Lab/TransfusionSvc/012704.asp) [ABO Only](http://www.childrensmn.org/Manuals/Lab/TransfusionSvc/012677.asp)ABR-ABO and Rh as part of other testing battery (Type and Screen, Newborn Workup, etc)ARC-ABO and Rh Recheck |
| **Related****Documents** | TS 4.02 Making a 1.0% Patient Cell SuspensionTS 4.09 Grading and Interpretation of Gel Card ReactionsTS 4.35 Resolving an ABO Discrepancy |
|  |  |
| **Materials** | **Equipment** | **Reagents** | **Supplies** |
| * IH-Centrifuge L
* IH-Reader 24-Stp
 | * IH-Card ABO/D(DVI-)+Rev A1B
* IH- A1 and B Cells
* IH-LISS
 | * 10 x 75 mm test tubes
* BB pipettes 10μL, 50μL, and 1000 μL
* Tips
* 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.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.  |
| **Before** **You Begin** | 1. Confirm sample acceptability and review patient history per procedure.
 |
| **Procedure** |  |
|  | **Step** | Action |
|  | 1 | Prepare a 1% suspension of patient’s cells in IH-LISS solution by pipetting 1000μL into an appropriately labelled test tube and adding 10μL of patient packed red blood cells. |
|  | 2 | Inspect the IH-Card 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. |
|  | 3 | Label the IH-Card according to TS 4.07 Inspection and Labelling of Gel Cards. |
|  | 4 | Carefully remove the foil strip from the IH-Card. |
|  | 5 | Pipette 50 μL of the 1% patient red cell suspension into each of the first 4 gel micro well reaction chambers labelled A, B,D (VI-), and Ctl take care to maintain an air gap between the upper reaction chamber containing the cells and the gel column below containing the antisera. |
|  | 6 | Gently re-suspend the IH-Cell A1 and B reagent red blood cells, ensuring complete suspension of the cells. |
|  | 7 | Pipette 50 μL IH-Cell A1 into the well labeled A1 and 50 μL of IH-Cell B into the well labeled B (with the red background) taking care to maintain an air gap between the upper reaction chamber and the gel column below. |
|  | 8 | Pipette 50 μL plasma into each of the microwells labeled A1 and B (with red background). |
|  | 9 | Centrifuge the IH-Card(s) for the pre-programmed time and speed for the 24 card centrifuge head (10 minutes at 910 rpm). |
|  | 10 | Use the table under Interpretation to determine the ABO.

|  |  |
| --- | --- |
| **If**  | **Then** |
| there are any discrepancies in reaction pattern when compared to the table  | * Resolve the discrepancy, per TS 4.35 Resolving an ABO Discrepancy
* IF the discrepancy in the reaction can not be resolved the ABO must be interpreted as INCONLUSIVE
 |
| an infant < 4 months old types as an O Rh negative and may be a transfer from another facility. Contact the patient care unit and determine if the patient was transported in from another facility | * Contact the other facility BB regarding the

 infant’s original blood type.* If a discrepancy is noted between infants

 current blood type and the initial blood type, transfuse the infant with group O Rh Neg. red cells, AB FFP/Cryo, and AB Rh Neg. platelets* Add problem comment PRTO to the

 patient’s BAD file. |

 |
|  | 11 | Compare the current ABO results with any previous ABO 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
 |

 |
|  | 13 | Review the final record including a final clerical check of sample, label, request, and interpretation. |
|  | 14 | Dispose of all gel cards and pipettes used for the examinations in a biohazard waste container. |
|  |  |
| **Interpretation** | Agglutination seen as clumps of red cells caught in the gel matrix is a positive result and indicates the presence of the corresponding antigen (forward test and D antigen) or antibody (reverse test). No agglutination is observed as a smooth surfaced button at the bottom of the gel column. This is a negative result and indicates the absence of the corresponding antigen (forward test and D antigen) or antibody (reverse test).Following centrifugation, IH-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 will interfere with an accurate reading of the results. |
| **If the forward grouping reaction of patient cells with**  | **And the reverse grouping reaction of patient plasma/serum tested with** | **Then interpret the ABO as** |
| **Anti-A is** | **Anti-B is** | **A1 cells is** | **B cells is** |
| 0 | 0 | +/H or NT | +/H or NT | O |
| + | 0 | 0 or NT | +/H or NT | A |
| 0 | + | +/H or NT | 0 or NT | B |
| + | + | 0 or NT | 0 or NT | AB |
| 0 = No agglutination + = Graded agglutination H = Hemolysis NT= Not TestedAll other reaction patterns must be interpreted as Inconclusive unless resolved. |
| **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.
* A red blood cell concentration or suspension medium different from that recommended.
* 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 from patients who have received plasma expanders of high molecular weight may five false positive results.
* Fibrin, clots, particulates or other artifacts may cause some red blood cells to be 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 field could lead to misinterpretation.
* A weak reaction is not an expected result for antigen typing and may be indicative of a false positive or weak/partial expression of the antigen. Further investigations may be warranted per site–specific procedures.
* If red blood cells (pellet at the bottom of the microtube) are too low in concentration, they become difficult to visualize and in certain cases, a weak positive reaction can fail to be detected.
* Decreased ABO antibody reactivity may be seen in disease states, the elderly or infants, resulting in false negative reactions.
* Weak reactions may be obtained in ABO serum/plasma blood grouping and are valid results. Very weak ABO subgroup may not be detected with the Anti-A and Anti-B reagents used in this IH-Card
* The Anti-B reagent does not react with the acquired B antigen
* Very weak expressions of the D antigen may not be detected. If the detection of weak D samples is required, the samples producing negative results with this anti-D reagent should be further tested with and anti-D reagent known to detect weak D antigen expression (i.e. IH-Anti-D (RH1) Blend).
* The performance characteristics of this product with chemically modified frozen/thawed or enzyme-treated red blood cells have not been established.
 |
| **Result Reporting** | TS 5.6 Entering Results for ABO/Rh testing or for ABO/Rh RecheckTS 5.7 Entering Results for ABO Grouping Only |
| **References** | *Product Insert, IH-Card ABO/D(DVI-)+Rev A1,B, Bio-Rad Medical Diagnostics, current edition**Product Insert, IH-Cell A1,B, Bio-Rad Medical Diagnostics, current edition**Product Insert, IH-Card Neutral, Bio-Rad Medical Diagnostics, current edition**Product Insert, IH-LISS Solution, Bio-Rad Medical Diagnostics, current edition*  |
| **Approval****Workflow** | Transfusion Service/Laboratory Director |
|  |  |
| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 1 | S. Cassidy | 02/17/2023 | Initial Version |
|  | 2 | S. Cassidy | 08/02/2023 | Added steps to result DP on blood grouping |