|  |
| --- |
| **Antibody Screen by Gel** |
| **Purpose** | This procedure provides instructions for performing an indirect antiglobulin test (IAT) on patient samples for the detection of unexpected antibodies using gel technique. |
| **Policy Statements** | * Methods of testing shall detect clinically significant antibodies and shall include incubation at 37C

 preceding an antiglobulin phase using 2 cell reagent-screening cells.* When clinically significant antibodies are detected, additional testing shall be performed prior to

 the transfusion of red cell products in non-emergency situations.* If the patient has been transfused with blood or blood components containing allogenic red cells,

 or has been pregnant in the preceding 3 months, or if the history is uncertain or unavailable, then a sample collected from the patient within 3 days of the transfusion must be tested.* Patient < 4 months old: Repeat antibody screening may be omitted for the remainder of infant’s

 admission period or until the infant reaches the age of 4 months, whichever is sooner.  |
| **Definitions** | IAT-Indirect Antiglobulin Test |
| **Test Codes** | %AS-Antibody ScreenRPAS-Repeat Antibody ScreenCASC-Credit Antibody Screen |
| **Related****Documents** | [Antibody Screen](http://www.childrensmn.org/Manuals/Lab/TransfusionSvc/012706.asp) |
|  |  |
| **Materials** | **Equipment** | **Reagents** | **Supplies** |
| * IH- Incubator
* IH-Centrifuge
* IH-Reader 24-(STP only)
 | * IH-Card IgG Gel
* 0.6% IH-Cell I and II
 | * MLA pipette tips
* 50 μl and 25 μl MLA pipette or Biohit pipette
 |
|  |
| **Sample** | No special preparation of the patient is required prior to specimen collection. Blood should be collected and labeled according to approved policies and procedures. [Collection of Patient Specimens](http://www.childrensmn.org/Manuals/Lab/TransfusionSvc/012709.asp)Fresh serum or plasma collected with or without anticoagulants (EDTA) may be used in indirect antiglobulin procedures for antibody detection. Testing should be performed as soon as possible. Samples that cannot be tested immediately should be stored at 2-8°C or frozen.In the case of potential recipients of blood transfusion, specimens must be tested within 3 days of collection if the patient has been transfused pregnant in the preceding 3 months, or if the history is uncertain or unavailable.Hemolyzed and grossly icteric blood samples may cause difficulty in interpretation, and test results should be used with caution. Grossly lipemic samples containing particulates that clog the gel, as indicated by diffuse blotches of red blood cells in the microtube, may be clarified by centrifugation or filtration and retested. |
| **Quality Control** | Reagents must be evaluated each day of use with appropriate controls.TS 18.14 Performing Quality Control IH-Reader 24 or Manual System |
| **Before** **You Begin** | Review the following procedures as needed.[TS 4.7 Inspection & Labeling of Gel Cards](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLab%20Procedures%5CTS%204.7%20Inspection%20and%20labeling%20of%20gel%20cards.doc)[TS 4.9 Gel Interpretation](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLab%20Procedures%5CTS%204.9%20Grading%20and%20interpretation%20of%20gel%20card%20reactions.doc) |
| **Procedure** |  |
|  | **Step** | Action |
|   | 1 | Bring samples and reagents to room temperature (18-25°C) |
| 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 | Labe the IH-Card. TS 4.07 Inspection and Labeling of Gel Cards |
|  | 4 | 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.** |
| 5 | Gently re-suspend the IH-Cell I-II ensuring complete suspension of the cells. |
|  | 6 | Add 50 μL of each appropriate reagent red cell suspension to the correspondingly labeled microwell on the IH-Card, taking care to maintain the air gap between the cells in the upper reaction chamber. |
|  | 7 | Add 25 μL of plasma/serum to each of the microtubes being used for testing.**Note: Carefully dispense the red blood cell suspension and the serum/[plasma, avoiding contact of the pipette tip with the contents of the microtubes to prevent carryover.** |
|  | 8 | Place the IH-Cards in a 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 at the preset temperature of 37°±1°C. |
|  | 9 | Centrifuge the IH-Cards using the 24-card head in the IH-Reader 24 or IH-Centrifuge L at the preset time and speed of 10 minutes at 85 x G. |
|  | 10 | When centrifugation cycle is complete, the IH-Reader 24 will read card or remove the IH-Card and read the reactions according to TS 4.09 Grading and Interpretation of Gel Card Reactions. |
|  | 11 | Compare current test results with previous records. See Appendix A. |
|  | 12 |

|  |  |  |
| --- | --- | --- |
| **Antibody Screen is:** | **and red cells are**  | **then proceed to the following:** |
| Negative | Not needed | TS 2.10 Sample Storage and Retrieval |
| Required immediately | [TS 3.4 Crossmatch Process-Red Cell Orders for Patients over 4 months old](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5C3%20Processing%20Orders%5CTS%203.4%20Crossmatch%20Process-Red%20Cell%20Orders%20for%20Patients%20over%204%20months%20old.doc) |
| Positive | Not needed, or not needed urgently | [TS 4.38 Investigation of a Positive Antibody Screen](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLab%20Procedures%5CTS%204.38%20Investigation%20of%20a%20Positve%20Antibody%20Screen.DOC) |
| Urgently required | Consult with the ordering provider and a Pathologist |

 |
|  |  |
| **Interpretation** |  |
| **If the anti-IgG gel card microtube shows** | Then | Interpret Antibody Screen  |
| No hemolysis or no agglutination with complete sedimentation of all red cells to the bottom of microtube. | Antibodies not detected  | Negative |
| Hemolysis and/or agglutination of any strength | Antibodies are present | Positive |
| Agglutination of red blood cells as seen by clumps of cells caught within the gel column is a positive test and indicates the presence of an unexpected antibody (ies). To grade the reaction follow procedure TS 4.09 Grading and Interpretation of Gel Card Reactions. No agglutination is observed as a smooth surfaced button at the bottom of the gel column. This is a negative result and indicates that no unexpected antibody was detected.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 accurate reading of the results |
| **Limitations** | * Low frequency antigens may not always be resent on IH-Cell I-II. Therefore, negative reactions with the screening reagent red blood cells do not always indicate the absence of unexpected antibodies.
* Negative reactions indicate that the serum/plasma contains no detectable antibodies against one of the antigens present on the reagent red blood cells.
* Because some antibodies show dosage effect, the antigen density on the reagent red blood cells should be considered when evaluating the test results (homozygous or heterozygous expression). A heterozygous expression of the antigen may result in weak antibodies not being detected.
* In very rare cases, HLA-antigens within the product may lead to false positive reactions.
* The reactivity of the IH-Cells may decrease during the dating period and therefore should not be used after the expiration date. The rat of decrease in reactivity is partially dependent on individual donor characteristics that are neither controlled nor predicted by the manufacturer.
* Maintaining the air gap during pipetting and incubation is critical. Cells present in the column prior to adding serum/plasma may not come in contact with the test serum/plasma and may decrease the sensitivity of the test system.
* Erroneous an 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 or protein or blood samples from patients who have received plasma expanders of high molecular weight may give 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 t 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.
* In some instances of autoimmune hemolytic anemia, the antibody may be completely adsorbed into the erythrocytes and not detectable by the indirect antiglobulin test.
* Rouleaux may cause false positive results. Repeat by tube testing using saline replacement technique as needed.
 |
| **Alternate Procedure** | [TS 4.20 Antibody screening-tube testing](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5C4%20Patient%20Specimen%20Analysis%5CTS%204.20%20Antibody%20screening-tube%20testing.doc) |
| **Result Reporting** | TS 5.9 Entering Results for an Antibody Screening-Gel or Tube |
| **References** | 1. AABB Standards for Blood Banks and Transfusion Services, current edition
2. Package insert: IH-Card AHG Anti-IgG, Bio-Rad Medical Diagnostics GmbH, current edition
3. Package insert: IH-Card Anti-IgG, -C3d, Bio-Rad Medical Diagnostics GmbH, current edition
4. Package insert: IH-Cell I II, Bio-Rad Medical Diagnostics GmbH, current edition
 |
| **Appendices** | [Appendix A](#Appendix_A): Antibody Screen Results Comparison table |
| **Approval****Workflow** | Transfusion Service/Laboratory Director |
|  |  |
| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
| 3 | S Cassidy | 02/17/2023 | Updated procedure for new reagents and change format from PDF |

# Appendix A: Antibody Screen Results Comparison Table

|  |  |  |
| --- | --- | --- |
| **If the current antibody screen is negative** | **And if a previous antibody screen result was** | **Then** |
| Negative | * Negative
* Not found
 | * Record the results
 |
| * A history of clinically significant antibodies
 | * Reconfirm the patient sample
* Check the date of the antibody identification (Note: Most clinically significant antibodies should still be reactive if the previous identification is recent)
* Repeat the antibody screen by gel or tube testing if in doubt of the initial result
* Perform AHG crossmatch with antigen negative unit(s) to the historic antibodies [TS 4.24 Compatibility testing - AHG, gel testing](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLab%20Procedures%5CTS%204.24%20Compatibility%20testing%20-%20AHG%2C%20gel%20testing.doc)
* TYAS orders: AHG Crossmatch 1 antigen negative unit to historic antibodies.
 |
| Positive | * Negative
* Not found
 | * Refer to [TS 4.38 Investigation of a Positive Antibody Screen](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLab%20Procedures%5CTS%204.38%20Investigation%20of%20a%20Positve%20Antibody%20Screen.DOC)
 |
| * A history of clinically significant antibodies **AND** antibody identification studies **have been performed within the past 30** days **AND** there is **no** change in the testing reaction pattern
 | * Perform AHG crossmatches with antigen negative units [TS 4.24 Compatibility testing - AHG, gel testing](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLab%20Procedures%5CTS%204.24%20Compatibility%20testing%20-%20AHG%2C%20gel%20testing.doc)
* TYAS order: AHG Crossmatch 1 antigen negative unit
 |
| * A history of clinically significant antibodies **AND** antibody identification studies **have NOT been** performed within the past30 days **OR** there ischange in the testing reaction pattern
 | * Forward to reference lab for antibody identification studies. [TS 6.1 Reference lab testing](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5C6%20Reference%20Lab%20Testing%5CTS%206.1%20Reference%20lab%20testing.doc)
* Perform AHG crossmatches with antigen negative units [TS 4.24 Compatibility testing - AHG, gel testing](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5CLab%20Procedures%5CTS%204.24%20Compatibility%20testing%20-%20AHG%2C%20gel%20testing.doc)
* TYAS order: AHG Crossmatch 1 antigen negative unit.
 |
| * Infant less than 4 months old.
 | * Refer to Appendix A of [TS 3.1 Newborn Workup Process](file:///%5C%5Ckidsnet.childrenshc.org%5Cchcdfs%5Cdept%5C3%20Processing%20Orders%5CTS%203.1%20NewBorn%20Workup%20Process.doc)
 |