|  |  |  |  |
| --- | --- | --- | --- |
|  | **Antibody Screen**  BB.R.1003.4 | **Dept:**  | 324311 |
| **Dept Name** | Blood Bank |
| **Effective Date:** | Title21  |
| **Revised Date:** | Title21 |
| **Name & Title**: CLIA Laboratory Medical Director | **Contact:** | Julie Simmons/Christina Warren |
| **Signature:** | Refer to Title21 | **Date:** | **Title21** |

**1. General Procedure Statement:**

1. **Purpose:** The antibody screen is used to test the plasma/serum for clinically significant antibodies to comply with pretransfusion testing in order to select blood components that will not cause harm to the recipient and have acceptable survival when transfused. The antibody screen is used during pregnancy to detect clinically significant antibodies that could cause hemolytic disease of the newborn.
2. **Responsible Department/Scope:**

 Procedure owner/Implementer: Julie H. Simmons/Christina S. Warren

 Procedure prepared by: Julie H. Simmons

 Who performs procedure: Department staff/management

1. **Definitions:**

AHG: Anti human globulin

 DAT: Direct Antiglobulin Test

 IgG: Immunoglobulin G: Potentially clinically significant antibodies

 IgM: Immunoglobulin M

 Requisition: WakeOne requisition or order form or equivalent

 PCW: Patient Caution Window

 ABS: Antibody Screen

1. **Sections:**
2. Immediate Spin (IS) Tube Testing
3. Indirect Antiglobulin Phase (AHG) Tube Testing
4. Gel Testing (AHG)
5. Resulting in SCC – Joint Worksheet
6. Protocol
7. The antibody screen is performed as a part of the Type and screen and also includes ABO/Rh testing.
8. The type and screen sample is retained for future crossmatches if packed cells are needed.
9. For crossmatch purposes, the type and screen can routinely be used for three (3) days.
10. Patients that meet established criteria may qualify for a delayed crossmatch sample that can be used

for up to thirty (30) days. *Refer to Crossmatch Protocols.*

5. Patient’s that have a negative antibody screen and no history of clinically significant antibody may receive ABORh compatible packed cells after either an immediate spin serological crossmatch or an electronic crossmatch if two (2) ABORh types have been performed.

*Refer to Protocols: Crossmatch Protocols*

6. Patients with a positive antibody screen and/or a history of clinically significant antibody(ies) must receive antigen negative units with a full crossmatch.

*Refer to Protocols: Crossmatch Protocols*

7. All positive test results must be investigated for clinically significant antibodies (reactive at 37C and/or in the antiglobulin test):

*Refer to Protocols: Antibody Identification: General*

8. Patient with a positive antibody screen and/or history of clinically significant antibody(ies) will be routinely crossmatched with two(2) units of antigen negative blood if a Type and Screen is received. This guarantees at least two units are available for immediate release if needed. Crossmatch should be charged to patient.

9. Delays in obtaining compatible red blood cells resulting from clinically significant antibodies must be reported promptly to the nurse or physician responsible for the patient. This pertains to both in-house patients and out patients. Document the call on the antibody summary form.

*Refer to Protocols: Crossmatch Protocols*

10. Obstetric patients with clinically significant antibodies will have a titer performed.

*Refer to Specials: Titers*

11. Clinically insignificant antibodies generally demonstrate at immediate spin so the immediate spin is not routinely performed for the antibody screen.

12. Cold autoantibodies reactive at 37C and antiglobulin may be due to carry over from room temperature.

12.1 In these cases, prewarm techniques at 37C to AHG phases are indicated.

*Refer to: Specials: Adsorption and Prewarm Techniques: Section III*

12.2 To determine if cold autoantibodies are pathological, perform a thermal amplitude.

 *Refer to: Specials: Screen for Thermal Amplitude and Specificity of Cold Autoagglutinins*

13*. Ortho* IgG gel cards are used when testing in gel.

13.1 Ortho 0.8% red cells should not be kept out of the refrigerator for more than 8 hours and

 should be at room temperature before use. Do not store the 0.8% red cells at room

 temperature.

13.3 Reagent red cells that are approved for use in Ortho gel cards (Immucor or Biotest) should

 be at room temperature prior to use.

15. Methods

 15.1 Gel is the preferred routine method.

**2. Procedure: I. Immediate Spin (IS) Tube Testing**

Chemical Risk Assessment: None

Biological Risk Assessment: Low

Protective Equipment: Lab coat, gloves

Supplies: 10x75 tubes, pipets

Reagents: Screening Cells

Equipment: Centrifuge

Specimen Requirements:

Plasma: Anti-coagulated specimen (CPDA-1, CPD, ACD or EDTA)

 Serum: Clotted

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **1.0** | **Compare patient identification and required patient information on Blood Bank requisition, blood specimen and Blood Bank LIS before proceeding to test.***Refer to: BB.Protocol: Comparing Patient Identification Prior to Testing* |  |
| **2.0** | **Label (1)one 12x75 or 10x75mm tube with a minimum of first 3 initials of the patient’s last name and 1 or 2 or 3 to reflect the screening cell being tested.** *Refer to: BB.Protocol: Blood Bank Work Organization, Section 3* |  |
| **3.0** | **Add two drops of patient serum/plasma to each tube labeled in step 2 using Blood Bank plastic transfer pipets.** |  |
| **4.0** | **Add one drop of the 2-5 % cell suspension of each vial to each patient test tube using the Blood Bank plastic transfer pipet.** 4.1 Example: Screen Cell 1 – one drop into tube labeled with minimum of first three letters  of last name and #1. |  |
| **5.0** | **Mix well.**  |  |
| **6.0** | **Centrifuge the tubes for the IS time indicated on centrifuge. Examine for hemolysis.** |  |
| **7.0** | **Resuspend cell button carefully reading macroscopically for agglutination using agglutination lamp.** |  |
| **8.0** | **Grade and enter test reactions in computer system or during downtime record test results immediately on BB requisition or equivalent.***Refer to Routine: Grading of Positive and Negative Reactions.**Refer to Procedure IV: Entering Results in SCC.* |  |
| **9.0** | **Interpret results***Refer to Attachment 1: Interpretation of Test Results.* |  |

**2. Procedure: II: Indirect Antiglobulin Phase (AHG) Tube Testing**

Chemical Risk Assessment: None

Biological Risk Assessment: Low

Protective Equipment: Lab coat, gloves

Supplies: 10x75 tubes, pipets

Reagents: Screening Cells, Antiglobulin, Coombs Control Cells, LISS or PEG

Equipment: Centrifuge, Incubator, Cell washer

Specimen Requirements:

Plasma: Anti-coagulated specimen (CPDA-1, CPD, ACD or EDTA)

 Serum: Clotted

|  **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **1.0** | **Compare patient identification and required patient information on BB requisition, blood specimen and Blood Bank LIS before proceeding to test.***Refer to: BB.Protocol: Comparing Patient Identification Prior to Testing* |  |
| **2.0** | **Review Patient caution window to assist in determining method to use.** 2.1 The following computer codes are used to indicate the preferred method: Do gel  Do LISS Do PEG Do SP2.2 If no specific method is indicated, then SP/PEG is the current default method.  |  |
| **3.0** | **Label (1)one 12x75 or 10x75mm tube with a minimum of first 3 initials of the patient’s last name and 1 or 2 or 3 to reflect the screening cell being tested.** *Refer to: BB.Protocol: Blood Bank Work Organization, Section 3* |  |
| **4.0** | **Add two drops of patient serum/plasma to each tube labeled in step 2 using Blood Bank plastic transfer pipets.** |  |
| **5.0** | **Add one drop of the 2-5 % cell suspension of each vial to each patient test tube using the Blood Bank plastic transfer pipet.** 5.1 Example: Screen Cell 1 – one drop into tube labeled with minimum of first three letters  of last name and #1. |  |
| **6.0** | **Mix well.**  |  |
| **7.0** | **Select the method and follow specific instructions for that method.**

|  |  |
| --- | --- |
| **Method** | **Do** |
| **LISS** | a. Add two drops of potentiating medium LISS to each tube.b. Mix wellc. Incubate at 37°C ± 2°C for 15 to 30 minutes.d. Remove tubes from incubatore. Centrifuge for the calibrated time (IS calibrated time). f. Examine supernatant for hemolysis. g. Resuspend each cell button gently. h. Grade agglutination macroscopically using agglutination viewer. i. Record results.*Refer to Section IV: Results Reporting* |
| **PEG** | a. Add two drops of potentiating medium PEG to each tube.b. Mix wellc. Incubate at 37°C ± 2°C for 15 to 30 minutes.d. Do NOT centrifuge after removing from incubator.e. Examine for hemolysis( if present, may indicate ab/ag reaction). f. Record results.*Refer to Section IV: Results Reporting* |
| **Saline** | a. Incubate at 37°C ± 2°C for 30 to 60 minutes.b. Remove tubes from incubatorc. Centrifuge for the calibrated time (IS calibrated time). d. Examine supernatant for hemolysis. e. Resuspend each cell button gently. f. Grade agglutination macroscopically using agglutination viewer. g. Record results.*Refer to Section IV: Results Reporting* |
| **Solid Phase**  | 1. Refer to ECHO and NEO manuals for testing
2. Refer to Section for specifics on running the crossmatch on the instruments.
 |

 |  |
| **8.0** | **Wash the tubes 3-4 times with 0.9% saline or equivalent.** *Refer to Equipment Operations: Centrifuge Operation, VII Cell Washing Manual and Automated.*  |  |
| **9.0** | **Add 2 drops of anti-IgG to dry cell button.**9.1 There may be a need to use polyspecific antiglobulin reagents instead of  anti-IgG. In these cases, the instructions will be in the patient caution window.  |  |
| **10.0** | **Mix and centrifuge for the calibrated time posted on the centrifuge.** |  |
| **11.0** | **Resuspend and read macroscopically for agglutination.** 11.1 If serological reactions are suspicious, read test microscopically by placing  tube on tube reader under microscope.  |  |
| **12.0** | **Grade and enter results immediately in BB computer system or during downtime record on BB requisition or equivalent.** |  |
| **13.0** | **Add one (1) drop of 2-5% IgG-sensitized control cells to all negative tests. Repeat steps 10 to 12 (macroscopic reading only).**

|  |  |  |
| --- | --- | --- |
| **Interpretation** | **Result** | **Additional Direction** |
| INVALID | Weak agglutination or none.(1+ or weaker) | Repeat the test. |
| VALID | Agglutination (2+ or greater) | Report the result.  |

13.1 Interpret IgG sensitized control cells.  |  |
| **14.0** | **Grade and enter test reactions in computer system or during downtime record test results immediately on BB requisition or equivalent.***Refer to Routine: Grading of Positive and Negative Reactions.**Refer to Procedure iV: Entering Results in SCC.* |  |
| **15.0** | **Interpret results***Refer to Attachment 1: Interpretation of Results.**Refer to Attachment 2: Limitations of Antibody Screen Methods* |  |

**2. Procedure: III: Gel Testing (IgG)**

Chemical Risk Assessment: None

Biological Risk Assessment: Low

Protective Equipment: Lab coat, gloves

Supplies: 10x75 tubes, pipets, MTS-Anti-IgG card, Pipets: 25 µl, 50 µl, 10 µl, MTS Diluent 2

Reagents: 0.8% Ortho Screening Cells

Equipment: Ortho Workstation

Specimen Requirements:

Plasma: Anti-coagulated specimen (CPDA-1, CPD, ACD or EDTA)

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **1.0** | **Compare patient identification and required patient information on Blood Bank requisition, blood specimen and Blood Bank LIS before proceeding to test.***Refer to: BB.Protocol: Comparing Patient Identification Prior to Testing* |  |
| **2.0** | **Review patient caution window (PCW) to assist in determining method to use.**  |  |
| **3.0** | **Inspect the Anti-IgG CardTM to make sure foil is intact and card has not dried out.**3.1 Make sure there are no liquid bubbles in the upper chamber. a. If there are liquid bubbles in the upper chamber, then centrifuge the card in the  Ortho workstation centrifuge before using.  |  |
| **4.0** | **Label the Anti-IgG CardTM with appropriate patient small accession label, with patient full name, MR#, last name or, at minimum, the first 3 letters of the patient’s last name (and Screening cell number (1,2,3).***Refer to: BB.Policy: Blood Bank Work Organization, Section 3* |  |
| **5.0** | **Remove foil seal from the card (leave unused wells covered).** |  |
| **6.0** | **Add 50µl of each 0.8% screening cell suspension to the corresponding microtube using an appropriate MLA pipette.**6.1 Make sure there is an air gap in the microtube.  Air Gap No Air Gap6.2 The test must be repeated if the air gap is not present. |  |
| **7.0** | **Add 25µl patient plasma/serum to each microtube using an appropriate MLA pipette.**  |  |
| **8.0** | **Incubate at 37°C** ± 2°C **for 15 to 40 min.**8.1 Incubation is normally 15 minutes, but may be extended up to 40 min. |  |
| **9.0** | **Centrifuge the gel card at the preset conditions (10 minutes) of the manufacturer.** |  |
| **10.0** | **Read front/back of each microtube macroscopically by holding up to light or against a white back ground.**10.1 Enter reactions into computer or during downtime record reactions on BB  requisition or equivalent immediately.10.2 Refer to Section IV: Entering Results in SCC10.3 Cards that need to be reviewed should be sealed with tape or parafilm and placed  into the refrigerator at 2-8C for up to 24 hours. *Refer to Routine: Grading of Positive and Negative Reactions* |  |
| **11.0** | **Interpret results***Refer to Attachment 1: Interpretation of Results.**Refer to Attachment 2: Limitations of Antibody Screen Methods* |  |

**2. Procedure: IV: Entering Results in SCC**

Chemical Risk Assessment: None

Biological Risk Assessment: None

Protective Equipment: Lab coat, gloves

Supplies/Reagents/Specimen Requirements: NA

Equipment: SCC Computer system

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **SCC JOINT WORKSHEET** | **Go to Results>Joint Worksheet or Patient Test worksheet.*** 1. Select Build and worksheet that corresponds to test needed from drop down box.
	2. Answer Yes to Add tests, then Select tests and F12 to accept.
	3. Select correct Rack that has been QC’d (if does not default).
	4. Enter results sheet appears.
	5. Select the specimen type, media, time and temperature from the drop down boxes to reflect the testing performed. Refer to the table that follows.
	6. Enter test reactions performed into the appropriate column.

| **Column** | **Choices** | **Description** |
| --- | --- | --- |
| STYPE(Specimen Type | PLELAPAESLNA | PlasmaEluateAbsorbed PlasmaAbsorbed EluateSalineNot Applicable |
| MEDIA | NAGLPGLISLSP | Not ApplicableGelPEGLISSSalineSolid Phase |
| TIME | NTIS5, 10, 15, 30, 40,45 | Not TestedImmediate SpinNumber of minutes |
| TEMP | 37RTNT | 37 CRoom TemperatureNot Tested |
| 1/37, 2/37, 3/37Tubes 1,2,3 at 37 incubation | NT0 TO 4+HM | Not TestedAgglutination strengthHemolysisMixed Field |
| 1/AHG, 2/AHG, 3/AHGTubes 1,2,3 after antiglobulin | NT0 TO 4+HM | Not TestedAgglutination strengthHemolysisMixed Field |
| 1/CC, 2/CC, 3/CCTubes 1,2,3 after addition of Coombs cells | NT0 TO 4+ | Not TestedAgglutination strength |
| INTRPInterpretation  | NEGPOSINVLD | NegativePositiveInvalid |

* 1. Make interpretation.

*Refer to Attachment 1: Interpretation of Test Results* |  |
| **SCC Patient Results** | **Go to Patient>Orders>Results*** 1. Enter MRN of Patient and <Enter>
	2. Review Patient Caution Window and <Esc>.
	3. Double Click on Antibody Screen Test to be resulted.
	4. Enter results for each test.
	5. Select the specimen type, media, time and temperature from the drop down boxes to reflect the testing performed. Refer to the table that follows.
	6. Enter test reactions performed into the appropriate column.
1. Select NT if not tested.

| **Column** | **Choices** | **Description** |
| --- | --- | --- |
| STYPE(Specimen Type | PLELAPAESLNA | PlasmaEluateAbsorbed PlasmaAbsorbed EluateSalineNot Applicable |
| MEDIA | NAGLPGLISLSP | Not ApplicableGelPEGLISSSalineSolid Phase |
| TIME | NTIS5, 10, 15, 30, 40,45 | Not TestedImmediate SpinNumber of minutes |
| TEMP | 37RTNT | 37 CRoom TemperatureNot Tested |
| 1/37, 2/37, 3/37Tubes 1,2,3 at 37 incubation | NT0 TO 4+HM | Not TestedAgglutination strengthHemolysisMixed Field |
| 1/AHG, 2/AHG, 3/AHGTubes 1,2,3 after antiglobulin | NT0 TO 4+HM | Not TestedAgglutination strengthHemolysisMixed Field |
| 1/CC, 2/CC, 3/CCTubes 1,2,3 after addition of Coombs cells | NT0 TO 4+ | Not TestedAgglutination strength |
| INTRPInterpretation  | NEGPOSINVLD | NegativePositiveInvalid |

* 1. F12 to Accept Reactions.
	2. Adjust the ‘User’ number if necessary.
1. ‘User’ number defaults to 1 which will generate a charge.
2. ‘User’ number should be changed to 0 so that no charge will be generated (i.e. if resulting CAP samples )
 |  |

**3. Review/Revised/implemented:**

 All procedures must be reviewed as documented in the Document Control Protocol.

 All new procedures and procedures that have major revisions must be signed by the CLIA Director.

 All reviewed procedures and procedures with minor revisions can be signed by the designated section medical

 director or designee.

**4. Related Procedures:**

Routine: Grading Positive and Negative Reactions

**5. References**:

 References: Technical Manual, revised periodically.

**6. Attachments**:

 Attachment 1: Interpretation of Test Results

 Attachment 2: Limitations of Antibody Screen

**7. Revised/Reviewed Dates and Signatures:**

 See Archived Document Change Control

**Attachment 1: Interpretation of Test Results**

|  |  |  |
| --- | --- | --- |
| **Observation** | **Interpretation** | **Comments** |
| **Hemolysis or Agglutination in any phase** | **Positive** | This may indicate the presence of a clinically significant serological antibody. Further investigation is required:* Refer to antibody identification procedures.
 |
| **ABSENCE of agglutination and hemolysis in any phase of testing.** | **Negative** | This is a negative test result and indicates the plasma/serum does not contain antibodies directed at antigens present on this cell.  |
| **Mixed Field** | **Review Patient Clinical Information**  | Proceed with caution.The presence of fibrin, clots or particulates may result in some cells layering on the top of gel.Mixed field in the forward type may indicate a previous transfusion and potential developing antibody.  |

**Attachment 2: Limitations of Antibody Screen Methods**

**Limitations of Indirect Antiglobulin Phase (AHG) Tube Crossmatch**

1. Some weakly reactive examples of antibodies may produce serologically compatible crossmatches; however, the donor cells are truly incompatible.
2. Some ABO incompatibilities may not be detectable when the recipient's antibody is of low titer or the donor antigen is poorly expressed.
3. The presence of a high concentration of IgG paraproteins in the sample can neutralize the polyspecific anti-human globulin and lead to a false negative result in the antiglobulin test.
4. Rare antibodies, notably some anti-Jka or anti-Jkb may be detected only when polyspecific AHG is used and when active complement is present.

**Limitations of Gel Card Method**

1. Grossly hemolyzed, cloudy or contaminated samples or samples with presence of a clot, may cause false positive or false negative results.
2. Aged or hemolyzed specimens may cause weaker reactions compared to those obtained with fresh sample.
3. Abnormal concentrations of serum proteins, the presence of infused macromolecular solutions in the serum or plasma or the presence of Wharton’s jelly in cord blood samples may cause non-specific agglutination of the red blood cells. It is suggested that red blood cells be washed before performing the test.
4. Samples with high-potency antibodies may coat the red blood cells completely, causing spontaneous agglutination.
5. If poorly anticoagulated plasma or incompletely clotted serum is used, fibrin residues may trap non-agglutinated red blood cells at the top of the gel, appearing as a pinkish or reddish layer. Although the results could be correctly interpreted, in a negative reaction the false appearance of a mixed field could lead to a misinterpretation in case of incompletely clotted serum samples, it is recommended to re-clot the serum and repeat the test.
6. No single method is able to detect all unexpected antibodies. The optimum reaction conditions may vary for different antibody specificities.
7. The presence of a high concentration of IgG paraproteins in the sample can neutralize the polyspecific anti-human globulin and lead to a false negative result in the antiglobulin test.
8. Rare antibodies, notably some anti-Jka or anti-Jkb may be detected only when polyspecific AHG is used and when active complement is present.
9. The Indirect Antiglobulin Test at 37 C in gel have been reported to show a lower level of sensitivity than results obtained with the tube technique, in the detection of weak agglutination reactions of the ABO system.
10. A false positive result in the Direct Antiglobulin Test can be due to the complement attached to red blood cells in specimens collected from infusion lines used to administer dextrose-containing solutions or in specimens collected in tubes containing silicone gel.
11. On occasions, unagglutinated red blood cells may be retained somewhere in the gel column with the appearance of very minute red dots or flecks. However, this nonspecific retention should not interfere with the interpretation of the results.

 **Limitations of Indirect Antiglobulin Phase (AHG) Tube and GEL Crossmatch**

1. In the Direct Antiglobulin test not all positive reactions indicate that clinically significant antibodies are present. Specific anti-IgG reagent and elution techniques may be used for additional investigation of positive results.
2. Nonspecifically adsorbed proteins (high dose IV immune globulin, multiple myeloma, autoimmune disorders and other diseases associated with elevated serum globulin ) and modification of red cell membrane by some drug, can cause positive Direct Antiglobulin test.
3. Red blood cell samples with a positive Direct Antiglobulin Test should not be used for Indirect Antiglobulin testing.
4. Antibody activity may decrease in the elderly, infants or persons with disease.