1. **General Procedure Statement:**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Antibody Identification** BB.Specials.1002.6 | **Dept:**  | **324311** |
| **Dept Name** | **Blood Bank** |
| **Effective Date:** | **12/2/2005** |
| **Revised Date:** | **Title 21** |
| **Name & Title: CLIA Laboratory Medical Director** | **Contact:** | **BB Management** |
| **Signature:**  | **Refer to Title 21** | **Date:** | **Title 21** |

1. **Purpose:** Identification of an antibody to red cell antigens requires testing the serum/plasma against a panel of selected red cell specimens with known antigen composition for the major blood groups.
2. **Responsible Department/Scope:**

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

 ii. Procedure prepared by: Julie H. Simmons

 iii. Who performs procedure: Department staff/management

1. **Definitions:**

SCC: Soft Computer Consultants, Blood Bank computer system

PCW: SCC Patient Caution Window

AHG: Anti human globulin

 DAT: Direct Antiglobulin Test

 IgG: Immunoglobulin G: Potentially clinically significant antibodies

 IgM: Immunoglobulin M

 Requisition: Wake One Requisition or equivalent

 PCW: Patient Caution Window

 Antigram: Print out of reagent red cells antigen typings by lot number

 Homozygous: Red cells that carry a double dose of antigen (i.e. EE)

 Heterozygous: Red cells that carry a single dose of antigen (i.e. Ee)

 Rouleaux: Aggregates of red cells that adhere to one another on their flat surface, giving a stack of coins

 appearance when viewed microscopically due to:



1) An in-vitro phenomenon resulting from abnormalities of serum protein concentration.

2) Heavier concentration of red cells with plasma/serum

3) Cold Autoagglutinates may appear as rouleaux

1. Antibody Identification Protocols

***Refer to Protocol Manual: Antibody Identification Protocol; BB.Protocol.1031***

1. Sections:

1. [Utilizing Antibody Identification Summary](#I)
2. [Tube Testing for Antibody Identification](#II)
3. [Gel Testing (AHG) for Antibody Identification](#III)
4. [Preparation of 0.8% Cell Suspension for Ortho Gel from 2-4% Cell Suspension (if 0.8% are not available)](#IV)
5. Testing with Enzymes
6. [Preparation of Enzyme Treated Cells](#VA)
7. [Enzyme Treated Cells – Testing in Tube](#VB)
8. Enzyme Treated Cells – Testing in Gel
9. [Antibody Identification and Ruling Out](#VI)
10. [Saline Replacement](#VII)
11. [Cold Antibody Identification](#VIII)
12. Tube Testing
13. Gel Testing
14. [Low Affinity Antibody Detection](#IX)
15. [Donath Landsteiner Test](#X)
16. [Chloroquine Treatment](#XI)
17. [Antigen Typing, Direct and Indirect](#XII)
18. Tube Testing
19. Gel Testing for Ortho Rh Phenotype (Gel Card)
20. Gel Testing for Antigen Typing using IgG Gel Card (Not monoclonal)
21. Gel Testing for Antigen Typing using MTS Buffered Gel Card (Monoclonal)
22. **Procedure**
23. **Utilizing Antibody Identification Summary Sheet**

Chemical Risk Assessment: None

Biological Risk Assessment: Low

Protective Equipment: Lab coat, gloves

Supplies: BB Requisition

Antibody Identification Summary sheet

Tan manila folder, tab on end

Reagents: None

Equipment: None

Specimen Type: None

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **1.0** | **Record all positive antibody screen reactions on Blood Bank requisition or equivalent.** |  |
| **2.0** | **Copy patient name and medical record number (MRN#) from specimen tube onto screen and/or panel Antigram sheets or use a patient label.** |  |
| **3.0** | **Fill out an Antibody Identification Summary sheet - (attachment 4) when:**1. A new antibody has been identified.
2. A previously identified antibody is still demonstrating and a selected screen is being performed.
3. When direct coombs testing is performed
4. When a patient has any discrepancy in ABO/Rh typing.

*Refer to Attachment 4: Antibody Identification Summary* |  |
| **4.0** | **Enter Patient identification: Place patient label or document patient information area provided in upper left corner of form.*** 1. Record Previous antibodies if present in BBIS: check patient computer file for documentation of previously identified antibodies.
 |  |
| **5.0** | **Enter Patient Information**5.1 Answer question: BMT at WFBMC, History in SCC PCW?:  Mark appropriate box based on information available.5.2 Answer question: History of Transfer from another facility?: If patient is a new admit, call floor/clinic and ask if patient was transferred to WFBMC from another facility.* 1. If so, mark "yes" and document name of facility in space provided along with name of person contacted at said facility.
	2. Check Care Everywhere in Wake One.
	3. If information is not available, mark "No".

5.3 Answer question: Transfused blood within 3 months in BBIS?: 1. Check computer file for patient transfusion history.
	* Mark "yes" if patient has been transfused within the last 3 months; indicate date.
	* Mark "no" if not transfused within the last 3 months.
	1. Obtain any transfusion history available from outside facility if applicable.
2. Mark "yes" if transfusion history obtained; indicate date.
3. Mark "not obtained" if not.
4. Mark “No” if not.
5. Medical Director and/or Pathology Resident may be able to obtain additional information.

5.5 Answer question: Pregnant within 3 months?: 1. If patient is female, obtain pregnancy history, mark appropriate box - "yes" or "no".

 If patient is male, mark "NA".* 1. Answer question: Patient received RHIG/WinRho?:
1. If antibody identified is NOT anti-D, record NA in box.
2. If antibody identified is anti-D, request information from patient's chart and mark "yes" if RHIG/WinRho were given and date given.
3. Mark "no" if neither product was given.
 |  |
| **6.0**  | **Record ABO & RH Typing**6.1Transcribe carefully any reactions from the original Blood Bank Requisition pertaining to patient ABO/Rh. Document rack #/instrument used in space provided. 6.2 Document interpretation of ABO/Rh based on reactions, include initials and date of tech performing current testing. |  |
| **7.0** | **Record DAT & Screening**7.1 Document reactions of all DAT testing in area provided. Specify Special Rack used. 7.2 Record interpretation of all testing performed.7.3 Document reactions of antibody screen. Include media used, incubation time, rack # /instrument used, if applicable, interpretation of screen, tech performing testing and date testing was performed. 7.4 Include any comments pertinent to the patient ABO/Rh in the "Comments” field. |  |
| **8.0** | **Record Antigen Typings**8.1 Record, with grade, all antigen typing performed on patient throughout current antibody work- up. 8.2 Record initials of tech performing testing.8.3 Document Delay in service notification: document name of person notified along with time and date of notification of delay in service due to extended antibody work-up. |  |
| **9.0** | **Fill out Charges/Reviews area**9.1 Fill out appropriate areas1. Tech Initiating/Date
2. Tech Completing/Date

*Note: Management and/or Medical Director will complete additional review sections including the Action “BOUT” (Professional Review Fee) and the initials of Reviewer.* |  |
| **10.0** | **Document Charges**10.1 Document if specimens have been sent off for Molecular antigen typing by marking the appropriate boxes.10.2 Mark all charges (ex. panels, selected screens, absorptions, elutions, antigen typings, etc.) applied to current work-up.*Refer to Attachment 7: ABID Charging Flow Chart* |  |
| **11.0** | **Place all completed requisitions and paperwork in a clear sleeve to be reviewed by management.** 11.1 Place in front desk box.  |  |
| **12.0** | **Create Patient antibody folders once work-up has been reviewed by management and is ready to be filed.** 12.1 Create a folder with the tan manila folders (tab on end of folder) located in the irradiator room by writing the patient’s last name, first name and medical record number on the end tab.1. This folder is created by the person filing the work-up.

12.2 File the folder in the irradiator room (alphabetically) and it will then be scanned to an electronic folder.12.3 Place reviewed workup in file.12.4 Place BB requisition in this folder once it has expired. |  |
| **13.0** | **Locate the file.**13.1 Search for the manila folder filed in alphabetic order.13.2 Search for the scanned file (when there is no longer a manila folder):1. On the “G” drive in the BB Shared folder under BB Historic Antibodies
2. They are also on the Blood Bank iShares site.

 *Refer to FD: Records Retention and Storage* |  |

1. **Tube Testing for Antibody Identific****ation**

 **(Immediate Spin is performed if cold antibody or IgM antibody is suspected)**

Chemical Risk Assessment: None

Biological Risk Assessment: Low

Protective Equipment: Lab coat, gloves

Supplies: 10x75mm test tubes

12x75mm test tubes

Blood Bank 6” Plastic Transfer Pipets

0.9% Saline or equivalent (PBS Buffered Saline or Blood Bank saline)

Reagents: 2-4% panel or screening Red Cells

2-5% IgG-sensitized control cells

Anti-IgG

PeG, LISS

Equipment: 37◦C incubator

Cell washer

Calibrated centrifuge

Macroscopic viewing mirror

Specimen Requirements: A properly labeled EDTA tube:

6.0 mL pink top is preferred

3.0 mL or 10.0 mL lavender top is also acceptable

A properly labeled clot tube, no serum separator, may be used if EDTA is not available.

Specimens are stored at 2-8◦C for 14 days.

Samples for crossmatching should be tested within 3 days of collecting.

***Refer to Specimen Labeling Requirements BB.FD.1001***

***Refer to Crossmatch Procedures, section V:Delayed Crossmatch Procedure at WFBMC and DMC BR, BB.ROUTINE.1007***

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **1.0** | **Compare patient name, medical record # and BBID # (if applicable) on the specimen label, Blood Bank requisition and computer.*** 1. Identifying information must be identical on ALL items.

*Refer to: Protocol: Comparing Patient Identification Prior to Testing (BB.PROTOCOL.1040)* |  |
| **2.0** | **Review patient’s SCC-PCW to assist in determining method to use.**2.1 The following computer codes are used to indicate the preferred method:  Do gel, Do LISS or Do PEG2.2 If no specific method is indicated, then Gel is the current default  methods.  |  |
| **3.0** | **Label (1)one 12x75 or 10x75mm tube for each reagent red cell to be tested with a minimum of first 3 initials of the patient’s last name and the reagent red cell number or auto for auto control .***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 3.0 using Blood Bank plastic transfer pipets.** |  |
| **5.0** | **Obtain reagent red cells to be tested and appropriate panel Antigram for lot number and/or the Antigen Plus antigram.**5.1 Record lot number/cell number from reagent red cell vial in the blank column  beside the reaction area for each vial.  |  |
| **6.0** | **Add one drop of the 2-5 % panel/screen cell vial (reagent red cell suspension) to each patient test tube using the manufacturer’s dropper.** 6.1 Verify that vial number and lot number match Antigram.  |  |
| **7.0** | **Mix well.** 7.1 Examine for hemolysis. |  |
| **8.0** |

|  |  |  |
| --- | --- | --- |
| **IS Needed?** | **Why?** | **Go to:** |
| **Yes** | **Cold or IgM antibody suspected** | **Proceed to Step 9.0** |
| **No** | **IgG only suspected** | **Proceed to Step 13.0** |

**Determine if an Immediate Spin (IS) Reaction is needed.** |  |
| **9.0** | **Centrifuge the tubes for the IS time indicated on centrifuge.** |  |
| **10.0** | **Remove tubes from centrifuge, no more than 3 at a time.**10.1 Examine for hemolysis. |  |
| **11.0** | **Resuspend cell button carefully reading macroscopically for agglutination using agglutination lamp.** |  |
| **12.0** | **Grade and record test results immediately on panel Antigram or equivalent.***Refer to Routine: Grading of Positive and Negative Reactions.* |  |
| **13.0** | **Follow specific instructions for method:**

|  |  |
| --- | --- |
| **Method** | **Do** |
| **PEG** | a. Add two drops of potentiating medium PEG to each tube.b. Mix wellc. Incubate at 37C ± 1°C (36°C to 38°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.g. Immediately proceed to wash phase. If any delay, re-incubate tubes at 37°C ± 1°C (36°C to 38°C) |
| **LISS** | a. Add two drops of potentiating medium LISS to each tube.b. Mix wellc. Incubate at 37°C ± 1°C (36°C to 38°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.j. Immediately proceed to wash phase. If any delay, re-incubate tubes at 37°C ± 1°C (36°C to 38°C) |
| **Saline** | a. Incubate at 37°C ± 1°C (36°C to 38°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.h. Immediately proceed to wash phase. If any delay, re-incubate tubes at 37°C ± 1°C (36°C to 38°C)) |

 |  |
| **14.0** | **Wash the tubes 3-4 times with 0.9% saline or equivalent decanting between washes after centrifugation.***Refer to Equipment Operations: Centrifuge Operation, VII Cell Washing Manual and Automated.*  |  |
| **15.0** | **Add 2 drops of anti-IgG to dry cell button.**Note: There may be a need to use polyspecific antiglobulin reagents instead of  anti-IgG. In these cases, the instructions will be in the computer file.  |  |
| **16.0** | **Mix and centrifuge for the calibrated time posted on the centrifuge.** |  |
| **17.0** | **Resuspend and read macroscopically for agglutination.** 17.1 Read suspicious test reactions microscopically (X10 magnification). |  |
| **18.0** | **Grade and record results immediately on Antigram or equivalent.***Refer to Routine: Grading of Positive and Negative Reactions.* |  |
| **19.0** | **Add one (1) drop of 2-5% IgG-sensitized control cells to all negative tests.**  |  |
| **20.0** | **Repeat steps 16 to18 (macroscopic reading only).** |  |
| **21.0** | **Interpretation of IgG sensitized control cells.**

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

 |  |
| **22.0** | **Interpretation of all phases.**

|  |  |  |
| --- | --- | --- |
| **Interpretation** | **Result** | **Additional Direction** |
| **Positive** | Hemolysis or agglutination in any phase of testing. | Record results on Antigram.Proceed to Identification and Ruling Out.  |
| **Negative** | Absence of agglutination or hemolysis in any phase of testing. | This is a negative test result and indicates a serologically compatible panel. |

 |  |

 **III:** **Gel Testing for Antibody Identification (AHG)**

Chemical Risk Assessment: None

Biological Risk Assessment: Low

Protective Equipment: Lab coat, gloves

Supplies: MTS-Anti-IgG card

 MTS – MTS Buffered (neutral) gel card if using enzyme treated cells

Pipets: 25 µl, 50 µl, 10 µl, 500 µl

MTS Diluent 2

Reagents: 0.8% Panel or Screening Red Cells

Equipment: Ortho Workstation

Specimen Requirements: A properly labeled EDTA tube:

6.0 mL pink top is preferred

3.0 mL or 10.0 mL lavender top is also acceptable

A properly labeled clot tube, no serum separator, may be used if EDTA is not available.

Specimens are stored at 2-8◦C for 14 days.

Samples for crossmatching should be tested within 3 days of collecting.

***Refer to Specimen Labeling Requirements BB.FD.1001***

***Refer to Crossmatch Procedures, section V:Delayed Crossmatch Procedure at WFBMC and DMC BR, BB.ROUTINE.1007***

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **1.0** | **Compare patient name, medical record # and BBID # (if applicable) on the specimen label, Blood Bank requisition and computer.**1.1 Identifying information must be identical on ALL items.*Refer to: Protocol: Comparing Patient Identification Prior to Testing (BB.PROTOCOL.1040)* |  |
| **2.0** | **Review patient’s SCC PCW to assist in determining method to use.** 2.1 The following computer codes are used to indicate the preferred crossmatch Method for testing patient: Do gel Do LISS Do PEG Do SP 2.2 If no specific method is indicated, then Gel is the current default method.  |  |
| **3.0** | **Inspect the Anti-IgG Card to make sure foil is intact and card has not dried out.** |  |
| **4.0** | **Label the Anti-IgG Card with appropriate patient information and reagent red cell number or auto.***Refer to: BB.Protocol: Blood Bank Work Organization, Section 3* |  |
| **5.0** | **Prepare 0.8% suspension of patient cells for autocontrol:**5.1 Dispense 1.0mL of MTS Diluent 2 to tube(s) using MTS Dispenser. *Refer to: BB.Protocol: Blood Bank Work Organization, Section 3*5.2 Remove 10µl of packed red blood cells from centrifuged EDTA tube.5.3 Mix well. Final suspension should be approximately 0.8%. |  |
| **6.0** | **Remove foil seal from the card (leave unused wells covered).** |  |
| **7.0** | **Add 50µl of each donor red blood cell unit suspension to the corresponding microtube using an appropriate MLA pipette.**7.1 Verify that vial number and lot number match Antigram.*7.2 Refer to Section IV: Preparation of 0.8% Cell Suspension from 2-4% Suspension if*  *0.8% reagent cells are not available.* |  |
| **8.0** | **Add 25µl patient plasma/serum to each microtube using an appropriate MLA pipette.**  |  |
| **9.0** | **Incubate at 37°C ± 2°C (35-39°C) for 15 to 40 min.**Note: Incubation is normally 15 to 40 minutes. Incubation should be extended to 40 minutes when using ‘made up’ 0.8% cells or when the screen reactions are ≤ 1+. |  |
| **10.0** | **Centrifuge the gel card at the preset conditions (10 minutes) of the manufacturer.** |  |
| **11.0** | **Read front/back each microtube macroscopically by holding up to light or against a white back ground and record reactions on Antigram or equivalent immediately.**Note: Cards are stable for 24 hours if properly sealed with tape and refrigerated at 1-6C.  *Refer to Routine: Grading of Positive and Negative Reactions.* |  |
| **12.0** | **Interpretation.**

|  |  |  |
| --- | --- | --- |
| **Observation** | **Interpretation** | **Comments** |
| Hemolysis or Agglutination in gel card  | **Positive** | Grade reaction and record result beside appropriate cell on Antigram.  |
| ABSENCE of Agglutination or Hemolysis gel card. | **Negative** | Record negative beside appropriate cell on Antigram. |

 |  |
| **13.0** | **Proceed to Section VI for Ruling Out and Antibody Identification.**  |  |

**IV.** **Preparation of 0.8% Cell Suspension for Ortho Gel from 2-4% Cell Suspension**

**(if 0.8% are not available)**

Chemical Risk Assessment: None

Biological Risk Assessment: Low

Protective Equipment: Lab coat, gloves

Supplies:12x75mm test tubes

Pipets: 25 µl, 50 µl, 10 µl, 500 µl

MTS Diluent 2

 Reagents: 2-4% Panel or Screening Red Cells

Equipment: Centrifuge

Specimen Type:

 Plasma: None

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **1.0** | Label appropriate number of 12x75mm test tubes with lot number, date and time prepared and cell number. * 1. Label large volumes of 0.8% cells (30-60 tests) to be used during multiple shifts with Attachment 6: Date Prepared label:

Manufacturer:Lot #/Cell#:Date/Time Prepared:Date/Time Expires (24hrs):Tech Who Prepared: 1. Complete label with manufacturer, lot and cell numbers, date/time prepared, date/time expires and initials of tech who prepared.
2. Place on calendar to remove cells after 24 hours

***Note: Expiration date is 24 hours after preparation.*** |  |

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **2.0** | Determine the number of tests needed.* 1. Using a fresh pipette tip each time, perform one of the following procedures.
	2. Inspect MTS diluent for discoloration, turbidity or contamination before use.

|  |  |
| --- | --- |
| **Number of tests needed** | **Make a 0.8% cell suspension** |
| **< 4 tests** | 1. Add 100 µl of 2-4% panel cells to the appropriately labeled tubes.
2. Add a few drops of MTS diluent 2 to each tube.
3. Centrifuge for 60 seconds.
4. Pipet off supernatant.
5. Add 200 µl of MTS diluent 2 to packed red cells, mix.
 |
| **< 30 tests** | 1. Centrifuge 2-4% reagent cells and remove supernatant.
2. Dispense 1.0 mL of MTS diluent 2 into labeled tubes.
3. Pipet 10 µl of the packed 2-4% panel cells to the appropriately labeled tubes.
4. Mix well.

*Note: volumes may be doubled for 60 tests.* |
|  |  |

 |  |
| **3.0** | Cells are ready for use. |  |
| **4.0** | Cover tubes with parafilm or cap and store prepared 0.8% cells in refrigerator for up to 24 hours. |  |

**V.** **Testing with Enzymes**

* + 1. **Preparation of Enzyme Treated Cells**

 Chemical Risk Assessment: low

 Biological Risk Assessment: low

 Protective Equipment: Lab coat, gloves

Reagents: 0.9% saline

 2-4% Reagent Red Cells

 Ficin Control from Immucor Panocell -10

 Ficin from Immucor Panocell -10 or GammaZyme-F

 Supplies: 10x75 mm or 12x75 mm glass test tubes

 Disposable pipettes

 Equipment: Macroscopic viewing mirror

Calibrated centrifuge

37◦C incubator

Specimen Requirements: A properly labeled EDTA tube:

6.0 mL pink top is preferred

3.0 mL or 10.0 mL lavender top is also acceptable

A properly labeled clot tube, no serum separator, may be used if EDTA is not available.

Specimens are stored at 2-8◦C for 14 days.

| **1:10 Dilution of Ficin from Immucor Panocell 10** | **GammaZyme-F** | **CHANGE /** **APPROVAL** |
| --- | --- | --- |
| 1. **Prepare a 1:10 dilution of the Ficin.**
	1. Label a 12x75 test tube Ficin Solution.
	2. Add 0.1mL of Ficin Solution to 0.9mL of saline.
	3. Mix well.
 | **1.0 Go to Step 2.**  |  |
| 2.0 **Label 3 sets of 10x75 or 12x75 tubes with the identifying information for the cells being** **treated.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Cells used** | **Label Testing** **set of tubes “T” AND:** | **Label Positive Control** **set of tubes “C pos” AND:** | **Label Negative Control** **set of tubes “C neg” AND:** |
| **Patient cells** | patient last name  | patient last name  | Patient last name  |
| **Donor units** | Last 5 digits of donor#  | Last 5 digits of donor #  | Last 5 digits of donor#  |
| **Reagent red cells** | cell # and lot #  | cell # and lot #  | Cell # and lot #  |

 |  |
| 1. **Wash red cells to be treated in 0.9% saline.**
	1. Donor units and patient cells should be washed three (3) times.
	2. Reagent red cells only need to be washed one (1) time.
 |  |
| **4.0 Resuspend the dry cell button in saline to a concentration of 3-4%.** |  |
| **5.0 Place one drop of 3-4% cells in corresponding labeled test tube.** |  |
| **6.0 Add 1 drop of 1:10 dilution of ficin to each**  **drop of 3-4% cell suspension. Refer to table**  **below.**  | **6.0 Add 1 drop of GammaZyme F to**  **each drop of 3-4% cell suspension.** **Refer to table below.** |  |
|

|  |  |  |  |
| --- | --- | --- | --- |
| **Cells used** | **Testing** **set of tubes**  | **Control (C POS)****set of tubes**  | **Negative Control (C NEG)****set of tubes**  |
| Patient cells | 1 drop of 3-4% cells 1 drop of enzyme | 1 drop of 3-4% cells 1 drop of enzyme | 1 drop of 3-4% cells 1 drop of enzyme |
| Donor units | 1 drop of 3-4% cells 1 drop of enzyme | 1 drop of 3-4% cells 1 drop of enzyme | 1 drop of 3-4% cells 1 drop of enzyme |
| Reagent red cells | 1 drop of 3-4% cells 1 drop of enzyme | 1 drop of 3-4% cells 1 drop of enzyme | 1 drop of 3-4% cells 1 drop of enzyme |

6.1 |  |
| **7.0 Incubate all tubes for 10-15 minutes at**  **37°C ± 1°C (36°C to 38°C).** | **7.0 Incubate all tubes for 10 minutes**  **at 37°C ± 1°C (36°C to 38°C).** |  |
| **8.0 Wash all tubes three times with**  **saline.** | **8.0 Wash all tubes three times with**  **saline.** |  |
| **9.0 Go to step 10.**  | **9.0 Resuspend the dry cell button**  **with 1 drop of saline.** 1. Cells are ready for testing.
 |  |
| **10.0 Test Control cells to determine if cells have been appropriately enzyme treated** 10.1 Add 2 drops of ficin control solution to “C pos” and 2 drops of saline to “C neg” tubes. 10.2 Mix well.10.3 Centrifuge tubes. a. Stop spin. b. Read immediately and interpret results without delay. c. Delay may result in dissociating antigen-antibody complexes leading to falsely  negative, and/or weakly positive reactions.  10.4 Gently suspend the red cell button and examine for agglutination.  10.5 Interpretation table below for “C pos” and “C neg” Control tests.

|  |  |  |
| --- | --- | --- |
| **Reaction** | **Enzyme Treatment** | **Next step** |
| **C pos** | **C neg** |  |  |
| **3+ to 4+** | 0 | Adequate enzyme treatment. (Not ficin overtreatment) | Passed proceed to step 11.  |
| **0 to 2+** | 1+ to 4+ | Inadequate enzyme treatment and/or ficin overtreatment. | Discard all tubes labeled T and C and repeat enzyme treatment procedure. |

 |  |
|  |
| **11.0 Proceed with “T” enzyme treated tests only if control tests above passed** *11.1Refer to V: Testing with Enzymes, Section B: Testing Enzyme Treated Cells in Tube*  *or Section C: Testing Enzyme Treated Cells in Gel* |  |
| **12.0 Enter Charges in SCC**12.1 Charge for enzyme panel.1. Patient, Orders, Modify
2. Add Action: BB Enzyme Panel (BENZP)
3. Go to Patient, Orders, Actions
4. Change Status to Confirmed
5. Change number of users to reflect the number of panels to charge.

12.2 Charge for extra enzyme treated cells.1. Patient, Orders, Modify
2. Add Action: BB Enzyme Tremnt (BENZT)
3. Go to Patient, Orders, Actions
4. Change Status to Confirmed
5. Change number of users to reflect the number of extra cells to charge.
 |  |

* + 1. **Testing Enzyme Treated Cells –in Tube**

Chemical Risk Assessment: None

Biological Risk Assessment: Low

Protective Equipment: Lab coat, gloves

Supplies: Tube Testing

10x75mm test tubes

12x75mm test tubes

Blood Bank 6” Plastic Transfer Pipets

0.9% Saline or equivalent (PBS Buffered Saline or Blood Bank saline)

Reagents: 0.8% or 3-4% Red Cells treated with 1:10 ficin or Gammazyme F

Equipment: 37◦C incubator

Cell washer

Calibrated centrifuge

Macroscopic viewing mirror

Specimen Requirements: A properly labeled EDTA tube:

6.0 mL pink top is preferred

3.0 mL or 10.0 mL lavender top is also acceptable

A properly labeled clot tube, no serum separator, may be used if EDTA is not available.

Specimens are stored at 2-8◦C for 14 days.

| **STEPS** | **INSTRUCTIONS** | **CHANGE /** **APPROVAL** |
| --- | --- | --- |
| **1.0** | **Compare patient name, medical record # and BBID # (if applicable) on the specimen label, Blood Bank requisition and computer.**1.1 Identifying information must be identical on ALL items.*Refer to: Protocol: Comparing Patient Identification Prior to Testing (BB.PROTOCOL.1040)* |  |
| **2.0** | **Remove the serum/plasma from the sample.**  |  |
| **3.0** | **Place the serum/plasma in a tube labeled with patient identifiers (First and last name and MRN and BBID number if used) and serum/plasma.** |  |
| **4.0** | **Label tubes for testing with patient name and cell number.** |  |
| **5.0** | **Add 2-3 drops of serum/plasma to each tube containing a drop of enzyme treated red cells labeled with patient name.***Note: Do not add PeG or LISS unless directed by management.**Note: Adding 3 drops of plasma/serum may enhance reactivity.* |  |
| **6.0** | **Add 1 drop of enzyme treated red cells to appropriate labeled tube.** |  |
| **7.0** | **Mix well gently.** |  |
| **8.0** | **Place all tubes in 37◦C +1◦C (36-38◦C) incubator for 15 to 30 minutes.**8.1 Gammazyme treated cells can be extended up to 60 minutes.  |  |
| **9.0** | **Remove all tubes from incubation and centrifuge at 3400±100 RPMs according to time stated on centrifuge.** |  |
| **10.0** | **Examine for hemolysis and agglutination.**10.1 Record reactions on appropriate antigram.  |  |
| **11.0** | **Wash tubes 3-4 times with saline decanting completely after each wash.** |  |
| **12.0** | **Add 2 drops of anti-IgG to each dry cell button.** |  |
| **13.0** | **Mix well.** |  |
| **14.0** | **Centrifuge at 3400 to 3600 rpms for AHG time stated on centrifuge.** |  |
| **15.0** | **Resuspend and examine macroscopically with viewing mirror for agglutination.** |  |
| **16.0** | **Grade and Record results on Antigram.**

|  |  |
| --- | --- |
| **Interpretation** | **Description** |
| **Positive** | Hemolysis or agglutination (weak, 1+, 2+, 3+ or 4+ or H) in any phase of testing may indicate the presence of an unexpected antibody. |
| **Negative** | The absence of agglutination and hemolysis in all phases of testing is a negative test result and indicates the serum does not contain antibodies directed at antigens present on the cells. |

 |  |
| **17.0** | **Confirm all negative reactions by adding 1 drop of IgG-sensitized control cells to all negative tubes.** |  |
| **18.0** | **Centrifuge at 3400 ±100 RPMs for the calibrated time stated on** **the centrifuge.** |  |
| **19.0** | **Resuspend and read for agglutination, macroscopically.** |  |
| **20.0** | **Grade and record control cell results on the antigram.**

|  |  |
| --- | --- |
| **Interpretation** | **Description** |
| **Valid** | After addition of IgG-sensitized cells to a negative test, the presence of agglutination (1+ or stronger) indicates that the anti-IgG was capable of reacting and that the negative antiglobulin test is valid. |
| **Invalid** | If IgG-sensitized cells added to confirm the activity of the anti-IgG show weak agglutination (<1+) or none after centrifugation, the test is invalid and must be repeated. |

 |  |
| **21.0** | **Enter Charges in SCC**21.1 Charge for enzyme panel.1. Patient, Orders, Modify
2. Add Action: BB Enzyme Panel (BENZP)
3. Go to Patient, Orders, Actions
4. Change Status to Confirmed
5. Change number of users to reflect the number of panels to charge.

21.2 Charge for extra enzyme treated cells.1. Patient, Orders, Modify
2. Add Action: BB Enzyme Tremnt (BENZT)
3. Go to Patient, Orders, Actions
4. Change Status to Confirmed
5. Change number of users to reflect the number of extra cells to charge.
 |  |

 **C. Testing Enzyme Treated Cells –in Gel**

Chemical Risk Assessment: None

Biological Risk Assessment: Low

Protective Equipment: Lab coat, gloves

Supplies: **MTS Buffered gel card (Neutral)**

Reagents: 0.8% Red Cells that have been enzyme treated. (Ortho or Immucor reagent cells)

Equipment: Ortho Workstation

Specimen Requirements: A properly labeled EDTA tube:

6.0 mL pink top is preferred

3.0 mL or 10.0 mL lavender top is also acceptable

A properly labeled clot tube, no serum separator, may be used if EDTA is not available.

Specimens are stored at 2-8◦C for 14 days.

| **STEPS** | **INSTRUCTIONS** | **CHANGE /** **APPROVAL** |
| --- | --- | --- |
| **1.0** | **Compare patient name, medical record # and BBID # (if applicable) on the specimen label, Blood Bank requisition and computer.**1.1 Identifying information must be identical on ALL items.*Refer to: Protocol: Comparing Patient Identification Prior to Testing (BB.PROTOCOL.1040)* |  |
| **2.0** | **Refer to the following sections to set up gel panel and prepare a 0.8% suspension using Immucor enzyme treated cells:**2.1 Section IV. Preparation of 0.8% Cell Suspension for MTS Gel from 2-4% Cell  Suspension. |  |
| **3.0** | **Remove the serum/plasma from sample.**  |  |
| **4.0** | **Place the serum/plasma in a tube labeled with patient identifiers (First and last name and MRN and BBID number if used) and serum/plasma.** |  |
| **5.0** | **Inspect the MTS BUFFERED Card to make sure foil is intact and card has not dried out.** |  |
| **6.0** | **Label the MTS BUFFERED Card with appropriate patient information and reagent red cell number or auto.***Refer to: BB.Protocol: Blood Bank Work Organization, Section 3* |  |
| **7.0** | **Remove foil seal from the card (leave unused wells covered).** |  |
| **8.0** | **Add 50µl of each donor enzyme treated 0.8% red blood cell unit suspension to the corresponding microtube using an appropriate MLA pipette.**8.1 Verify that vial number and lot number match Antigram.  |  |
| **9.0** | **Add 25µl patient plasma/serum to each microtube using an appropriate MLA pipette.** |  |
| **10.0** | **Incubate at 37°C ± 1°C (36-38°C) for 15 to 40 min in Ortho workstation incubator.**Note: Incubation is normally 15 to 40 minutes. Incubation should be extended to 40 minutes when using ‘made up’ 0.8% cells or when the screen reactions are ≤ 1+. |  |
| **11.0** | **Centrifuge the MTS BUFFERED gel card at the preset conditions (10 minutes) of the manufacturer.** |  |
| **12.0** | **Read front/back of each microtube macroscopically by holding up to light or against a white back ground and record reactions on Antigram or equivalent immediately.** Note: Cards are stable for 24 hours if properly sealed with tape and refrigerated at 1-6C in an upright position.  *Refer to Routine: Grading of Positive and Negative Reactions.* |  |
| **13.0** | **Interpretation.**

|  |  |  |
| --- | --- | --- |
| **Observation** | **Interpretation** | **Comments** |
| Hemolysis or Agglutination in gel card  | **Positive** | Grade reaction and record result beside appropriate cell on Antigram.  |
| ABSENCE of Agglutination or Hemolysis gel card. | **Negative** | Record negative beside appropriate cell on Antigram. |

 |  |
| **14.0** | **Proceed to Section VI for Ruling Out and Antibody Identification.** |  |

**VI:** **Antibody Identification and Ruling Out**

Chemical Risk Assessment: None

Biological Risk Assessment: Low

Protective Equipment: Gloves

Supplies: None

Reagents: None

Equipment: None

Specimen Requirements: None

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **1.0** | **Obtain the antigram for the antibody panel that is being used.** * 1. Confirm that the lot # and exp. date of the panel and antigram match.
 |  |
| **2.0** | **Record the following information on the antigram:**1. Patient name and MRN (written or use label)
2. Date of testing
3. Initials of testing tech
4. Method used (PeG, LISS, Gel, Saline)
5. Incubation time
6. Phases read (37 **º**C, IgG, check cells (CC), Gel, Immediate Spin(IS))
7. Sample used: Plasma or Eluate
8. Any special testing conditions (cold panel, ficin treated cells, etc..)
9. Write the lot # and cell # for each cell used in column next to reactions.
 |  |
| **3.0** | **Grade and record the results of the panel in the appropriate phase in the spaces provided.** |  |
| **4.0** | **If there are no positive reactions, consult with management or charge tech.** |  |
| **5.0** | **Rule out using negative cells.**5.1 Rule out all clinically significant antibodies that are antigen homozygous on the negative cells. 5.2 Mark through the antibodies at the top of the sheet as they are ruled out with homozygous cells.5.4 If there are no homozygous cells available and the antibody can only be ruled out using heterozygous cells, make a note above the antigen profile for that antibody. 1. Testing incubation time must be at maximum to rule out heterozygously.
 |  |
| **6.0** |  **Identify the antibody present by matching the pattern of positive and negative cells with the antigen profiles on the sheet.** 6.1 As a general rule, 3 antigen positive cells that react and 3 antigen negative cells that do not react are needed to identify each specific antibody. 6.2 Utilize the screening cell reactions also to rule out or confirm. 6.3 Run additional cells to confirm the identity of the antibody if needed with at least one negative cell if no additional rule out cells are needed. Refer to step 7.6.4 Record the interpretation of the antibody identification on the line at the top of the Antigram.6.5 Complete an Antibody Identification Summary Sheet if new antibody.   *Refer to Attachment 4: Antibody Identification Summary sheet.*  |  |
| **7.0** | **Rule out all additional antibodies by searching for other cells using Antigen Plus that are negative for the suspected antibody and homozygous for the antigen that needs to be ruled out.** 7.1 Complete documentation on Antigram. *Refer to Specials: Antigen Plus* |  |
| **8.0** | **Perform a cold panel if the results of the panel are inconclusive or negative and there are reactions at immediate spin in the crossmatch and/or the reverse group is positive in a cell that does not correspond to the forward blood type.**8.1 Test at room temperature and 4**º**C.*Refer to Section VIII: Cold Antibody Identification* |  |
| **9.0** | **Note: If the results of the panel are inconclusive and it is not possible to rule out all the clinically significant antibodies, consult with the manager.**9.1 Other testing may be requested by management.*Refer to other sections in this procedure: Section V: Testing with Enzyme;* *Section IX: Low Affinity Antibody Detection; Section X:Donath Landsteiner Test.**Refer to Special procedures: Adsorption and Prewarm Techniques;* *Screen for Thermal Amplitude and Specificity of Cold Autoagglutinins.* |  |

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **10.0** | **Type the patient for the antigen corresponding to the identified antibody(ies) unless the patient was recently transfused (within previous 3 months).** 10.1 Grade your results. 10.2 Note if mixed field, which makes the results inconclusive. 10.3 Check if patient has been RBC genotyped and rule in/out based on these  results whether patient transfused or not. 10.4 Do a complete Rh phenotype with Rh antibodies (if needed).a. Type with anti-C, anti-E and anti-c. b. Type for anti-e only if positive with anti-E. c. Test for both alleles for MN and Lewis antigens when these antibodies are suspected.d. Record the results in the space on the antibody identification summary. |  |
| **11.0** | Investigate all positive autocontrols, even if the panel is negative.11.1 Perform a DAT.  *Refer to Special Procedures: Direct Antiglobulin Test*11.2 Suspect an autoantibody if the autocontrol is positive and the panel is  positive with all cells. Additional techniques may be required to rule out  clinically significant antibodies in the presence of a warm or cold  autoantibody.  *Refer to Specials: Adsorption and Prewarm Techniques; Acid Elution*11.3 Suspect an antibody to a component in the reagent cells (such as a drug)  when only the same manufacturer’s cells are reactive and different  manufacturer’s cells or auto control are nonreactive.  |  |
| **12.0** |  Crossmatch antigen negative units through AHG as needed. *Refer to Routine: Crossmatch Protocols for crossmatch requirements for patients with antibodies.* |  |
| **13.0** | Complete documentation on Antigram.Refer to Section VIII: Cold Antibody Identification, Step 5 |  |

**VII:** **Saline Replacement**

Chemical Risk Assessment: None

Biological Risk Assessment: Low

Protective Equipment: Lab coat, gloves

Supplies: Blood Bank 6” Plastic Transfer Pipets

0.9% Saline or equivalent (PBS Buffered Saline or Blood Bank saline)

Reagents: None

Equipment: Centrifuge

Macroscopic viewing mirror

Microscope

Specimen Requirements: A properly labeled EDTA tube:

6.0 mL pink top is preferred

3.0 mL or 10.0 mL lavender top is also acceptable

A properly labeled clot tube, no serum separator, may be used if EDTA is not available.

Specimens are stored at 2-8◦C for 14 days.

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **1.0** | **Recentrifuge the plasma or serum /cell mixture of the tube(s) that appear to show rouleaux microscopically**. |  |
| **2.0** | **Remove tube(s) carefully from centrifuge to avoid resuspending.** |  |
| **3.0** | **Pipet the plasma/serum from the tube while leaving the cell button undisturbed.** 3.1 SAVE the plasma when there is not enough plasma available to be replaced after reading.  |  |
| **3.0** | **Replace plasma with an equal amount of saline and mix well.** 1. 2 drops saline usually
 |  |
| **4.0** | **Centrifuge the mixture.** |  |
| **5.0** | **Remove the tube from the centrifuge and gently resuspend the cell button.**  |  |
| **6.0** | **Observe for agglutination** 6.1 Read macroscopically with viewing mirror and microscopically and record results on antigram. 6.2 Note saline replacement technique used on BB requisition or antigram. |  |
| **7.0** | **Interpretation after saline replacement:**

|  |  |  |
| --- | --- | --- |
| **Result** | **Interpretation** | **Next steps** |
| **Agglutination** | True agglutination | Continue to panel for antibody identification. |
| **No agglutination** | Rouleaux | Document rouleaux.Put SCC message in PCW. |

 |  |

**VIII:** **Cold Antibody Identification**

Chemical Risk Assessment: None

Biological Risk Assessment: Low

Protective Equipment: Lab coat, gloves

Supplies: Test tubes

Specimen rack

Pipettes

Reagents: 2-4% screening cells and panel cells

Isotonic saline

Equipment: Calibrated centrifuge

Macroscopic viewing mirror

Refrigerator

Specimen Requirements: A properly labeled EDTA tube:

6.0 mL pink top is preferred

3.0 mL or 10.0 mL lavender top is also acceptable

A properly labeled clot tube, no serum separator, may be used if EDTA is not available.

Specimens are stored at 2-8◦C for 14 days.

A. Tube Method

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **1.0** | **Compare patient name, medical record # and BBID # (if applicable) on the specimen label, Blood Bank requisition and computer.**1.1 Identifying information must be identical on ALL items.*Refer to: Protocol: Comparing Patient Identification Prior to Testing (BB.PROTOCOL.1040)* |  |
| **2.0** | **Review patient’s ABO Type to determine additional A or B cells that need to be tested with the patient’s serum/plasma.**

|  |  |
| --- | --- |
| **Patient’s Group** | **Additional Cells to Be Tested** |
| A | A1 and A2 cells |
| B | B cells |
| AB | A1, A2 and B cells |

 |  |
| **3.0** | **Label (1)one 12x75 or 10x75mm tube for each reagent red cell to be tested with a minimum of first 3 initials of the patient’s last name and the reagent red cell number.*** 1. Cells to include:
1. a cord cell (i) or an adult I negative cell
2. an auto control
3. panel cells
4. cells mentioned in Step 2.0 according to patient’s blood type
 |  |
| **4.0** | **Add two drops of patient serum/plasma to each tube labeled in step 2 using Blood Bank plastic transfer pipets.** |  |
| **5.0** | **Obtain reagent red cells to be tested and appropriate Antigram for lot number.**5.1 Record the following information on the antigram:1. Patient name and MRN (written or use label)
2. Date of testing
3. Initials of testing tech
4. Incubation time
5. Phases read (Immediate Spin(IS), room temperature (RT), 1-6 **º**C)
6. Sample used: Plasma/Serum or Eluate
7. Any special testing conditions (i.e. cold panel)
8. Write the lot # and cell # for each cell used in column next to reactions.
 |  |
| **6.0** | **Add one drop of the 2-5 % reagent red cell suspension to each patient test tube.** |  |
| **7.0** | **Mix well.**  |  |
| **8.0** | **Incubate for 15 – 30 minutes at room temperature (20-24◦C).** |  |
| **9.0** | **Centrifuge the tubes for the IS time indicated on centrifuge.** |  |
| **10.0** | **Remove tubes from centrifuge one at a time.**10.1 Examine for hemolysis. |  |
| **11.0** | **Resuspend cell button carefully reading macroscopically for agglutination using agglutination viewing mirror.** |  |
| **12.0** | **Grade and record test results immediately on panel Antigram or equivalent.***Refer to Routine: Grading of Positive and Negative Reactions.* |  |
| **13.0** | **Incubate tubes for 15-30 minutes at 1-6◦C by placing in refrigerator.** Note: Some antibodies react preferentially at 15-18**◦**C so an additional phase can be included if needed. |  |
| **14.0** | **Repeat step 9.****Caution: Do not allow tubes to warm to room temperature after centrifugation** |  |
| **15.0** | **Place spun tubes back in refrigerator or in cold bath (4-6C) for 5 minutes prior to reading for agglutination.** |  |
| **16.0** | **Repeat steps 11 -12.** |  |
| **17.0** | **Interpretation of all phases.**

|  |  |  |
| --- | --- | --- |
| **Interpretation** | **Result** | **Additional Direction** |
| **Positive** | Hemolysis or agglutination in any phase of testing. | Record results on Antigram.Proceed to Identification and Ruling Out. |
| **Negative** | Absence of agglutination or hemolysis in any phase of testing. | This is a negative test result and indicates a serologically compatible test. |

 |  |
| **18.0** | **Consider autoantibody if all cells are reactive including the autocontrol.**18.1 Cold autoantibodies may react with all cells making it difficult to detect  alloantibodies. 18.2 Additional techniques may be necessary to remove the cold autoantibody. a. Prewarm Technique b. Cold Adsorption *Refer to Specials: Adsorption and Prewarm Techniques*  |  |
| **19.0** | **Complete documentation on Antigram.**19.1 Refer to Step 5.  |  |
| **20.0** | **Enter Charges in LIS**20.1 Charge for cold panel in SCC.1. Patient, Orders, Modify
2. Add Action: BB Cold Panel (BCLDP)
3. Go to Patient, Orders, Actions
4. Change Status to Confirmed
5. Change number of users to reflect the number of panels to charge.

  |  |

**VIII: Cold Antibody Identification**

Chemical Risk Assessment: None

Biological Risk Assessment: Low

Protective Equipment: Lab coat, gloves

Supplies: **MTS Buffered gel card (Buffered)**

Reagents: 0.8% Red Cells.

Equipment: Ortho Workstation

Specimen Requirements: A properly labeled EDTA tube:

6.0 mL pink top is preferred

3.0 mL or 10.0 mL lavender top is also acceptable

A properly labeled clot tube, no serum separator, may be used if EDTA is not available.

Specimens are stored at 2-8◦C for 14 days.

**B. Gel Method**

| **STEPS** | **INSTRUCTIONS** | **CHANGE /** **APPROVAL** |
| --- | --- | --- |
| **1.0** | **Compare patient name, medical record # and BBID # (if applicable) on the specimen label, Blood Bank requisition and computer.**1.1 Identifying information must be identical on ALL items.*Refer to: Protocol: Comparing Patient Identification Prior to Testing (BB.PROTOCOL.1040)* |  |
| **2.0** | **Review patient’s ABO Type to determine additional A or B cells that need to be tested with the patient’s serum/plasma.**

|  |  |
| --- | --- |
| **Patient’s Group** | **Additional Cells to Be Tested** |
| A | A1 and A2 cells |
| B | B cells |
| AB | A1, A2 and B cells |

 |  |
| **3.0** | **Inspect the MTS BUFFERED Card to make sure foil is intact and card has not dried out.** |  |
| **4.0** | **Label MTS Buffered gel card for each reagent red cell to be tested with a minimum of first 3 initials of the patient’s last name and the reagent red cell number.*** 1. Cells to include:
1. a cord cell (i) or an adult I negative cell
2. an auto control
3. panel cells
4. cells mentioned in Step 2.0 according to patient’s blood type
 |  |
| **5.0** | **Refer to the following sections to set up gel panel and prepare a 0.8% suspension if needed:**5.1 Section IV. Preparation of 0.8% Cell Suspension for Gel from 2-4% Cell  Suspension. |  |
| **6.0** | **Remove the serum/plasma from sample.**  |  |
| **7.0** | **Place the serum/plasma in a tube labeled with patient identifiers (First and last name and MRN and BBID number if used) and serum/plasma.** |  |
| **8.0** | **Remove foil seal from the card (leave unused wells covered).** |  |
| **9.0** | **Add 50µl of each donor 0.8% red blood cell unit suspension to the corresponding microtube using an appropriate MLA pipette.**9.1 Verify that vial number and lot number match Antigram. |  |
| **10.0** | **Add 25µl patient plasma/serum to each microtube using an appropriate MLA pipette.** |  |
| **11.0** | **Incubate at RT (20 to 24 C) or 1-6C for 15 to 30 min.**Note: Incubation is normally 15 minutes, but may be extended up to 40 min.11.1 If both RT and 1-6C testing is needed, then a separate card will need to be set up for  each incubation temperature. |  |
| **12.0** | **Centrifuge the MTS BUFFERED gel card at the preset conditions (10 minutes) of the manufacturer.** |  |
| **13.0** | **Read front each microtube macroscopically by holding up to light or against a white back ground and record reactions on Antigram or equivalent immediately.** Note: Cards are stable for 24 hours if properly sealed with tape and refrigerated at 1-6C in an upright position.  *Refer to Routine: Grading of Positive and Negative Reactions.* |  |
| **13.0** | **Interpretation.**

|  |  |  |
| --- | --- | --- |
| **Observation** | **Interpretation** | **Comments** |
| Hemolysis or Agglutination in gel card  | **Positive** | Grade reaction and record result beside appropriate cell on Antigram.  |
| ABSENCE of Agglutination or Hemolysis gel card. | **Negative** | Record negative beside appropriate cell on Antigram. |

 |  |
| **14.0** | **Proceed to Section VI for Ruling Out and Antibody Identification.** |  |

**IX:** **Low Affinity Antibody Detection (All testing is completed at 1-6◦C.)**

Chemical Risk Assessment: None

Biological Risk Assessment: Low

Protective Equipment: Lab coat, gloves

Supplies: Test tubes

Specimen rack

Pipettes

Reagents: 2-4% screening cells and panel cells (1-6◦C)

Anti-IgG (1-6◦C)

IgG-sensitized control cells

EluWash (1-6◦C Working Wash from Elution kit)

Isotonic saline

Equipment: Calibrated centrifuge

Macroscopic viewing mirror

Refrigerator

Specimen Requirements: A properly labeled EDTA tube:

6.0 mL pink top is preferred

3.0 mL or 10.0 mL lavender top is also acceptable

A properly labeled clot tube, no serum separator, may be used if EDTA is not available.

Specimens are stored at 2-8◦C for 14 days.

Eluate

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **1.0** | **Compare patient name, medical record # and BBID # (if applicable) on the specimen label, Blood Bank requisition and computer.**1.1 Identifying information must be identical on ALL items.*Refer to: Protocol: Comparing Patient Identification Prior to Testing (BB.PROTOCOL.1040)* |  |
| **2.0** | **Bring all reagents and specimens to 1-6◦C prior to testing.** |  |
| **3.0** | **Label (1)one 12x75 or 10x75mm tube for each reagent red cell and auto control to be tested with a minimum of first 3 initials of the patient’s last name and the reagent red cell number or AC for auto control.***Refer to: BB.Protocol: Blood Bank Work Organization, Section 3* |  |
| **4.0** | **Add two drops of 1-6◦C patient serum/plasma/eluate to each tube labeled in step 3 using Blood Bank plastic transfer pipets.** |  |
| **5.0** | **Obtain 1-6◦C reagent red cells to be tested and appropriate Antigram for lot number.**5.1 Record the following information on the antigram:1. Patient name and MRN (written or use label)
2. Date of testing
3. Initials of testing tech
4. Incubation time
5. Phases read (Immediate Spin(IS), 1-6 **º**C, IgG, check cells (CC))
6. Sample used: Plasma or Eluate
7. Any special testing conditions (i.e. low affinity testing at 1-6 **º**C)
8. Write the lot # and cell # for each cell used in column next to reactions.
 |  |
| **6.0** | **Add one drop of the 2-5 % cell suspension to each corresponding test tube.** 1. reagent red cells
2. patient cells (auto control)
 |  |
| **7.0** | **Centrifuge on the IS setting and then put at 1-6◦C prior to reading.** |  |
| **8.0** | **Remove tubes one at a time.**8.1 Examine for hemolysis. |  |
| **9.0** | **Resuspend cell button carefully reading macroscopically for agglutination using agglutination lamp.** |  |
| **10.0** | **Record results on requisition or antibody identification summary or Antigram.** |  |
| **11.0** | **Incubate 30± 5 (25-35) minutes at 1-6◦C in refrigerator.** |  |
| **12.0** | **Remove from refrigerator.** |  |
| **13.0** | **Centrifuge on the IS setting.** |  |
| **14.0** | **Gently resuspend tubes.**14.1 Check for hemolysis and agglutination. |  |
| **15.0** | **Record results on Antigram or equivalent.**  |  |
| **16.0** | **Wash 4 times with EluWash (1-6◦C Working Wash from Elution kit)** |  |
| **17.0** | **Add 2 drops of 1-6◦C anti-IgG and mix.** |  |
| **18.0** | **Centrifuge on AHG setting.** |  |
| **19.0** | **Resuspend and read macroscopically for agglutination.** 19.1 If serological reactions are suspicious, read test microscopically by placing  tube on tube reader under microscope.  |  |
| **20.0** | **Grade and record results immediately on Antigram or equivalent.***Refer to Routine: Grading of Positive and Negative Reactions.* |  |
| **21.0** | **Add one (1) drop of 2-5% IgG-sensitized control cells to all negative tests.** 19.1 Repeat steps 18 to 20 (macroscopic reading only). |  |
| **22.0** | **Interpretation of IgG sensitized control cells.**

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

 |  |
| **23.0** | **Interpretation of all phases.**

|  |  |  |
| --- | --- | --- |
| **Interpretation** | **Result** | **Additional Direction** |
| **Positive** | Hemolysis or agglutination in any phase of testing. | Record results on Antigram.Proceed to Identification and Ruling Out. |
| **Negative** | Absence of agglutination or hemolysis in any phase of testing. | This is a negative test result and indicates a serologically compatible test. |

 |  |
| **24.0** | **Enter Charges in LIS**24.1 Charge for cold panel in SCC.1. Patient, Orders, Modify
2. Add Action: BB Cold Panel (BCLDP)
3. Go to Patient, Orders, Actions
4. Change Status to Confirmed
5. Change number of users to reflect the number of panels to charge.

*Refer to Attachmen7: ABID Charging Flow Sheet* |  |

**X.** **Donath Landsteiner (DL) Test**

Chemical Risk Assessment: Low

Biological Risk Assessment: Low

Protective Equipment: Lab coat, gloves

Reagents: Freshly collected normal serum known to lack unexpected antibodies, to be used as a source of complement.

 A 50% suspension of washed group O red cells that express the P antigen.

Supplies: 10x75 mm or 12x75 mm glass test tubes

 Disposable pipettes

 Form: Donath-Landsteiner Test worksheet

Equipment: Light magnifying lamp

 Centrifuge

 Ice Bath 0° C

 37°C Incubator

Specimen Requirements:

Patient **serum** separated from a freshly collected blood sample maintained at 37°C until tested.

 Allow specimen to clot at 37°C to avoid loss of antibody by autoadsorption before testing.

 Normal serum fresh with negative antibody screen. This sera is used for complement.

 Cell suspension Group O that expresses P antigen. These cells can be obtained from segments from

 blood units. Unit cell must have a negative DAT.

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **1.0** | **Prepare 50% suspension of Group O cells from Group O segments.** **Note:** The P antigen is a high incidence antigen and is expressed on nearly all red cells. Cells of the P1 (P1+) or P2 (P1-) phenotypes will also synthesize the P antigen; therefore, a cell typing positive or negative with P1 antisera would be an acceptable choice.* 1. Perform DAT on unit cells and record results on worksheet.
1. DAT must be negative.
	1. Wash approximately 0.5ml (10 drops) of packed cells in a 12x75 tube a minimum of 3 times with saline.
	2. Resuspend washed red cells with an equal volume (10 drops) of saline to obtain a 50% suspension.
 |  |
| **2.0** | **Prepare Normal Serum fresh.**2.1 Do antibody screen using PeG procedure. a. Antibody screen must be negative.***Refer to Antibody Screen Indirect Antiglobulin Test Tube and Gel Methods; BB.ROUTINE.1004.1***  |  |
| **3.0** | **Set up the test.**3.1 Label 3 sets of 10 x 75mm test tubes. a. A-1, A-2, A-3 b. B-1, B-2, B-3 c. C-1, C-2, C-3

|  |  |  |  |
| --- | --- | --- | --- |
| To each tube listed: | Add patient’s serum | Add fresh normal serum | Add 50% P+ cells |
| **A-1, A-2****B-1, B-2****C-1, C-2** | 10 drops |  | 1 drop |
| **A-2, A-3****B-2, B-3****C-2, C-3** |  | 10 drops | 1 drop |

3.2 Mix all tubes well.  |  |
| **4.0** | **Incubate the tubes as follows:**

|  |  |
| --- | --- |
| **For Tubes labeled:** | **Do:** |
| **A** | 1. Incubate tubes in a bath of melting ice, placed in a 1-6°C refrigerator for 30 minutes.
2. After 30 minutes, remove tubes from refrigerated ice bath.
3. Place them in a 37°C incubator for 60 minutes.
 |
| **B** | * 1. Incubate tubes in a bath of melting ice, placed in

1-6°C refrigerator for 90 minutes.  |
| **C** | * 1. Incubate tubes in a 37°C incubator for 90 minutes
 |

  |  |
| **5.0**  | **Remove ALL tubes from incubation after appropriate interval.** |  |
| **6.0** | **Mix gently.** |  |
| **7.0** | **Centrifuge ALL tubes for 60 seconds at 3500±100 RPM's.** |  |
| **8.0** | **Record results on worksheet.** *Refer to Attachment 1: Donath-Landsteiner Test Worksheet.* |  |
| **9.0** | **Interpret results.**9.1

|  |
| --- |
| **Interpretation** |
| **Positive DL Test** | **Negative DL Test** | **Inconclusive** |
| Hemolysis ONLY in A-1 and A-2 |  | Hemolysis in any tube other than A-1 and A-2 |
| NO hemolysis in A-3,B-1, B-2, B-3, C-1, C-2, C-3 | NO hemolysis in any tubes  |  |

 |  |
| **10.0** | **Enter into computer system.**10.1 Order Charge only test: DL in SCC.10.2 Order and result test in SCC:1. Patient, Orders, Modify
2. Add test: DLT
3. Result under: Patient, Orders, Results
	1. Enter positive or negative result and select interpretation
	2. Add comment
 |  |
| **11.0** | **Send out to Blood Center of Wisconsin for DL testing for confirmation.**11.1 DL test is currently a laboratory developed test (DLT). |  |

**XI.** **Chloroquine Treatment**

Chemical Risk Assessment: Low

Biological Risk Assessment: Low

Protective Equipment: Lab coat, gloves

Reagents:Gamma-Quin (Chloroquine diphosphate solution)

 Blood Bank Saline

Supplies: 12x75 mm glass test tubes

 Disposable pipettes

Equipment: Light magnifying lamp

 Calibrated centrifuge

37◦C incubator

Cell washer

Specimen Requirements:

Anticoagulated blood is preferred. The specimen should be tested as soon as possible after collection.

If delay in testing should occur, the specimen must be stored at 1° to 10°C. Blood drawn into EDTA should not be stored for longer than seven days. It is best to test oxalated or heparinized blood samples within two days of being drawn. Clotted specimens are suitable, but difficulty may be experienced in obtaining the required volume of red blood cells for the procedure. Red blood cells from clots, or from samples collected into ACD or CPD, may be treated up to 21 days after collection.

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **1.0** | **Compare patient name, medical record # and BBID # (if applicable) on the specimen label, Blood Bank requisition and computer.**1.1 Identifying information must be identical on ALL items.*Refer to: Protocol: Comparing Patient Identification Prior to Testing (BB.PROTOCOL.1040)*  |  |
| **2.0** | **Label two (2) 12 x 75 mm glass tube with Patient Name and MRN number or use one of the printed patient labels.**  |  |
| **3.0**  | **Prepare the red blood cells to be treated by washing them at least three times in large volumes of saline to remove contaminating human serum or plasma.** 3.1 Place a minimum of 0.5 mL of packed red blood cells into one of the  properly labeled **12 x 75 mm** glass tube.* 1. Fill tube ¾ full with saline.
	2. Centrifuge for at least 60 seconds at 3300 to 3500 rpms.
	3. Remove supernatant by pipetting.
	4. Repeat steps 3.2 to 3.4 two (2) more times to have at least three (3) washes completed prior to treatment.

NOTE: If the treated red blood cells are needed for warm autoadsorption, a larger volume will be required, and the volume of Gamma-Quin should be adjusted accordingly. The ratio of reagent to washed, packed red blood cells should be 4:1. |  |
| **4.0** | **Place 10 drops (approximately 0.5 mL) of the washed, packed red blood cells in the second labeled test tube from step 2.0.** |  |
| **5.0** | **Add 40 drops (approximately 2.0 mL) of Gamma-Quin to the 10 drops of washed, packed red blood cells. The ratio is 4:1.**5.1 Mix thoroughly. |  |
| **6.0** | **Incubate for thirty (30) minutes to two hours at room temperature** **(20°C-26°C).** 6.1 Perform a DAT at 30 minute intervals to monitor the progress of the  dissociation of antibody.a. The time taken for dissociation varies between patients. b. Thirty minutes should be regarded as the minimum time within which  significant dissociation of immunoglobulin can be expected to occur. c. Do not extend the treatment beyond two hours. |  |
| **7.0** | **Wash the red blood cells at least three times in large volumes of saline to remove the chloroquine solution.**7.1 Centrifuge the tube containing the chloroquine and red cells.7.2 Remove the supernatant.* 1. Fill tube ¾ full with saline.
	2. Centrifuge for at least 60 seconds at 3300 to 3500 rpms.
	3. Remove supernatant by pipetting.
	4. Repeat steps 7.3 to 7.5 two (2) more times to wash a total of three (3) times.

NOTE: Slight hemolysis may be observed with some specimens, but this may be ignored. NOTE: More than three washes may be required if the test is carried out in a smaller test tube than that recommended. |  |
| **8.0** | **Resuspend the red blood cells to a concentration of 3-4% in saline for further testing.** 8.1 If the red blood cells are to be used for warm autoadsorption, they may be left packed.8.2 The red blood cells may be treated with an enzyme before being used for  autoadsorption, if desired.8.3 The red blood cells may be used for antigen typing. Go to Section XII.*NOTE: Antigen typings on chloroquine-treated red blood cells must be interpreted cautiously, as reactions may be somewhat weaker than with untreated red blood cells, even when using high-protein or antiglobulin-reactive reagents that meet FDA potency requirements.* **Saline-reactive (whether made from IgM or chemically modified IgG) or monoclonal blood grouping reagents should not be used for antigen typings on chloroquine-treated red blood cells, as reactions may be markedly weaker than expected, giving rise to the possibility of false negative results.** |  |
| **9.0** | **Perform Quality Control by performing a DAT on the red blood cells after treatment if not done in Step 6.1.**9.1 The DAT will not become negative in all cases, but may be sufficiently  reduced in strength by the treatment to enable the treated red blood cells to  be used effectively for warm autoabsorption.* 1. The chloroquine dissociation procedure may not be successful in all cases, but consistent failure of the treatment to reduce the strength of the direct antiglobulin test on in vivo coated red blood cells from different patients may be an indication of product deterioration.
	2. Consult with management after performing DATs.
 |  |
| **10.0** | **Proceed to antigen typing (ONLY if DAT IgG is negative) or adsorption procedures *(reduced strength DAT treated cells acceptable for adsorption process)* as necessary.***Refer to Specials: Adsorptions and Prewarm Techniques* |  |
| **11.0** | **Charge for treating cells in SCC**11.1 Charge for extra enzyme treated cells.1. Patient, Orders, Modify
2. Add Action: BB Chemical (TRT)
3. Go to Patient, Orders, Actions
4. Change Status to Confirmed
5. Change number of users to reflect the number of cells treated.
 |  |

1. **Antigen Typing: Direct and Indirect**

 **A. Tube Testing for Antigen Typing**

Chemical Risk Assessment: Low

Biological Risk Assessment: Low

Protective Equipment: Lab coat, gloves

Reagents:Antisera being tested (Commercial and Unlicensed)

 Positive (Heterozygous) Cell and Negative Cell for Controls

 Blood Bank Saline

 AHG

 Coombs Control Cells

Supplies: 10 x 75 or 12x75 mm glass test tubes

 Disposable pipettes

 Forms: Antigen Typing Worksheet (BB.FORMS.1003), WBFH Antigen Typing Card (donor units)

Equipment: Light magnifying lamp

 Calibrated centrifuge

 37◦C incubator

Specimen Requirements:

Anticoagulated blood is preferred. The specimen should be tested as soon as possible after collection.

If delay in testing should occur, the specimen must be stored at 1° to 10°C. Blood drawn into EDTA should not be stored for longer than seven days. It is best to test oxalated or heparinized blood samples within two days of being drawn. Clotted specimens are suitable, but difficulty may be experienced in obtaining the required volume of red blood cells for the procedure. Red blood cells from clots, or from samples collected into ACD or CPD, may be treated up to 21 days after collection.

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **1.0** | **Compare patient identification and required patient information on requisition and blood specimen before proceeding to antigen typing for patients or confirm donor number for donor samples.** *Refer to: Protocol: Comparing Patient Identification Prior to Testing (BB.PROTOCOL.1040)* |  |
| **2.0**  | **Verify that the patient has not been transfused with red blood cells during the previous 3 months.** |  |
| **3.0** | **Check to see if autocontrol or DAT was performed on the specimen.**Note: Red blood cells with a positive DAT cannot be used in the indirect antiglobulin test for antigen typing. |  |
| **4.0** | **Check the Antigen Typing Rack in SCC to see if the antisera has had quality control performed within the past 24 hours.**4.1 Proceed to testing in step 6.0 if quality control has been performed within the past 24 hours.4.2 Proceed to next step if quality control has not been performed.  |  |
| **5.0** | **Select reagent red cells to be used for quality control.**5.1 Select a Positive control which is a cell heterozygous for antigen. a. Example: Positive control for anti-E should be a cell positive for E and e.5.2 Select a Negative control which is a cell negative for antigen. a. Example: Negative control for anti-E should be a cell negative for E. |  |
| **6.0** | **Label tubes with donor unit # or patient identification.** 6.1 Label controls when needed with positive (plus vial number) or negative  control (plus vial number). |  |
| **7.0** | **Follow manufacturer’s directions for specific antisera. Remember to:**7.1 Check to see if antiglobulin procedure is required for indirect testing.7.2 Verify the DAT is negative for indirect testing1. Do not use DAT positive samples for indirect testing.

7.3 Wash cells if required by manufacturer.7.4 Test with media indicated for unlicensed antisera. |  |
| **8.0** | **Complete testing according to manufacturer’s directions for specific antisera.** *Refer to Direction Circular Notebook or Bench Direction Circular Notebook for detailed instructions for testing with each antisera. .*  |  |
| **9.0** | **Grade and record results in computer or during downtime record on Antigen Typing Worksheet for donor units or Patient Summary Sheet for patient or Rare Antisera QC if QC was performed.***Refer to: Attachment 2: BB.FORMS.1081 Rare Antisera Quality Control**Refer to: Attachment 3: BB.FORMS.1003 Antigen Typing Worksheet**Refer to: Attachment 4: BB.FORMS.1195 Antibody Identification Summary* |  |
| **10.0** | **Interpretation of Controls if performed.**

|  |  |  |
| --- | --- | --- |
| **Control** | **Acceptable** | **Not Acceptable** |
| **Positive**  | Reaction strength of ≥1+ | Reaction strength < 1+ or Nonreactive |
| **Negative**  | Nonreactive | Reactive any strength |

10.1 Repeat any testing performed and controls if not acceptable.10.2 Report to management any antisera that repeats unacceptable and remove  from use.  |  |
| **11.0** | **Complete a blue WFBH Antigen Typing Card for donor units and place the SCC antigen label that prints out on the blue card .***Refer to Attachment 5: WFBH Antigen Typing Card (Blue)*11.1 Carefully verify antigen typing label to unit and place on the blue card. |  |

 **B. Gel Testing for Ortho Rh Phenotype (Gel Card)**

Chemical Risk Assessment: Low

Biological Risk Assessment: Low

Protective Equipment: Lab coat, gloves

Reagents:Positive (Heterozygous) Cell and Negative Cell (4% ±1%) for Controls, MTS Diluent 2 Plus

 Rh phenotype gel card

Supplies: 50 ul, 10 uL pipettes

 Forms: Antigen Typing Worksheet (BB.FORMS.1003), WBFH Antigen Typing Card (donor units)

Equipment Diluent Dispenser, Ortho Workstation

Specimen Requirements:

Anticoagulated blood is preferred. The specimen should be tested as soon as possible after collection.

If delay in testing should occur, the specimen must be stored at 1° to 10°C. Blood drawn into EDTA should not be stored for longer than seven days. It is best to test oxalated or heparinized blood samples within two days of being drawn. Clotted specimens are suitable, but difficulty may be experienced in obtaining the required volume of red blood cells for the procedure. Red blood cells from clots, or from samples collected into ACD or CPD, may be treated up to 21 days after collection.

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **1.0** | **Compare patient identification and required patient information on requisition and blood specimen before proceeding to antigen typing for patients or confirm donor number for donor samples.** *Refer to: Protocol: Comparing Patient Identification Prior to Testing (BB.PROTOCOL.1040)* |  |
| **2.0**  | **Verify that the patient has not been transfused with red blood cells during the previous 3 months.** |  |
| **3.0** | **Check to see if autocontrol or DAT was performed on the specimen.**Note: Red blood cells with a positive DAT cannot be used in the indirect antiglobulin test for antigen typing. |  |
| **4.0** | **Check the rare antisera worksheets to see if the Rh phenotype card lot number has had quality control performed within the past 24 hours.**4.1 Proceed to testing in step 6.0 if quality control has been performed within the  past 24 hours.* 1. Proceed to next step if quality control has not been performed.
 |  |
| **5.0** | **Select reagent red cells to be used for quality control. These should be 4% ±1% cell suspensions. Do NOT use 0.8% cells suspensions. Card and cells should come to 18-25C before use.** 5.1 Select a Positive control which is a cell heterozygous for antigen. a. Example: Positive control for anti-E should be a cell positive for E and e.5.2 Select a Negative control which is a cell negative for antigen. a. Example: Negative control for anti-E should be a cell negative for E. |  |
| **6.0** | **Label card with donor unit # or patient identification.** 6.1 Label controls when needed with positive (plus vial number) or negative  control (plus vial number). |  |
| **7.0** | **Prepare a 4% ±1% red blood cell suspension.**7.1 Dispense 0.5 mL of MTS Diluent 2 Plus into a clean, labeled test tube. 7.2 Dispense 25uL of packed red blood cells into the same test tube. |  |
| **8.0** | **Dispense 10uL of the 4% suspension into the appropriate well of the gel card.** |  |
| **9.0** | **Centrifuge gel card(s) in the Ortho centrifuge for 10 minutes.**9.1 After centrifugation remove the gel card(s) and read the results. |  |
| **10.0** | **Grade and record results in computer or during downtime record on Antigen Typing Worksheet for donor units or Patient Summary Sheet for patient or Rare Antisera QC if QC was performed.***Refer to: Attachment 2: BB.FORMS.1081 Rare Antisera Quality Control**Refer to: Attachment 3: BB.FORMS.1003 Antigen Typing Worksheet**Refer to: Attachment 4: BB.FORMS.1195 Antibody Identification Summary* |  |
| **11.0** | **Interpretation of Controls if performed.**

|  |  |  |
| --- | --- | --- |
| **Control** | **Acceptable** | **Not Acceptable** |
| **Positive**  | Reaction strength of ≥2+ | Reaction strength < 2+ or Nonreactive |
| **Negative**  | Nonreactive | Reactive any strength |

11.1 Repeat any testing performed and controls if not acceptable.11.2 Report to management any antisera that repeats unacceptable and remove  from use.  |  |
| **12.0** | **Complete a blue WFBH Antigen Typing Card for donor units and place the SCC antigen label that prints out on the blue card .***Refer to Attachment 5: WFBH Antigen Typing Card (Blue)*12.1 Carefully verify antigen typing label to unit and place on the blue card. |  |

**C. Gel Testing for Antigen typing using IgG Gel Card**

 **(Anti-Fya, Anti-Fyb – Not monoclonal)**

Chemical Risk Assessment: Low

Biological Risk Assessment: Low

Protective Equipment: Lab coat, gloves

Reagents:Positive (Heterozygous) Cell and Negative 0.8% Cell for Controls, MTS Diluent 2

 Appropriate IgG gel card

Supplies: 50 ul, 10 uL pipettes

 Forms: Antigen Typing Worksheet (BB.FORMS.1003), WBFH Antigen Typing Card (donor units)

Equipment Diluent Dispenser, Ortho Workstation

Specimen Requirements:

Anticoagulated blood is preferred. The specimen should be tested as soon as possible after collection.

If delay in testing should occur, the specimen must be stored at 1° to 10°C. Blood drawn into EDTA should not be stored for longer than seven days. It is best to test oxalated or heparinized blood samples within two days of being drawn. Clotted specimens are suitable, but difficulty may be experienced in obtaining the required volume of red blood cells for the procedure. Red blood cells from clots, or from samples collected into ACD or CPD, may be treated up to 21 days after collection.

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **1.0** | **Compare patient identification and required patient information on requisition and blood specimen before proceeding to antigen typing for patients or confirm donor number for donor samples.** *Refer to: Protocol: Comparing Patient Identification Prior to Testing (BB.PROTOCOL.1040)* |  |
| **2.0**  | **Verify that the patient has not been transfused with red blood cells during the previous 3 months.** |  |
| **3.0** | **Check to see if autocontrol or DAT was performed on the specimen.**Note: Red blood cells with a positive DAT cannot be used in the indirect antiglobulin test for antigen typing. |  |
| **4.0** | **Check the rare antisera worksheets to see if the antisera has had quality control performed within the past 24 hours.**4.1 Proceed to testing in step 6.0 if quality control has been performed within the  past 24 hours.* 1. Proceed to next step if quality control has not been performed.
 |  |
| **5.0** | **Select reagent red cells to be used for quality control.**5.1 Select a Positive control which is a cell heterozygous for antigen. a. Example: Positive control for anti-E should be a cell positive for E and e.5.2 Select a Negative control which is a cell negative for antigen. a. Example: Negative control for anti-E should be a cell negative for E. |  |
| **6.0** | **Review the manufacturer’s insert for the antisera being used to confirm procedure.** |  |
| **7.0** | **Visually inspect gel card, then label card with donor unit # or patient identification.** 6.1 Label controls when needed with positive (plus vial number) or negative  control (plus vial number).6.2 Label with antisera being tested. |  |
| **8.0**  | **Remove the foil seal from the MTS Anti-IgG Card. Use within 1 hour of removal.**  |  |
| **9.0** | **Add 25 uL of the antisera to the appropriate reaction chamber of the opened well.** |  |
| **10.0** | **Dispense 50uL of the 0.8% suspension into the appropriate well of the gel card.**10.1 Observe that the contents of the reaction chamber(s) are combined. If necessary tap  GENTLY. |  |
| **11.0** | **Incubate at 37C±2C for 15 minutes.** |  |
| **12.0** | **Centrifuge gel card(s) in the for 10 minutes.**12.1 After centrifugation remove the gel card(s) and read the front/back for agglutainationresults. |  |
| **10.0** | **Grade and record results in computer or during downtime record on Antigen Typing Worksheet for donor units or Patient Summary Sheet for patient or Rare Antisera QC if QC was performed.***Refer to: Attachment 2: BB.FORMS.1081 Rare Antisera Quality Control**Refer to: Attachment 3: BB.FORMS.1003 Antigen Typing Worksheet**Refer to: Attachment 4: BB.FORMS.1195 Antibody Identification Summary* |  |
| **11.0** | **Interpretation of Controls if performed.**

|  |  |  |
| --- | --- | --- |
| **Control** | **Acceptable** | **Not Acceptable** |
| **Positive**  | Reaction strength of ≥2+ | Reaction strength < 2+ or Nonreactive |
| **Negative**  | Nonreactive | Reactive any strength |

11.1 Repeat any testing performed and controls if not acceptable.11.2 Report to management any antisera that repeats unacceptable and remove  from use.  |  |
| **12.0** | **Complete a blue WFBH Antigen Typing Card for donor units and place the SCC antigen label that prints out on the blue card .***Refer to Attachment 5: WFBH Antigen Typing Card (Blue)*12.1 Carefully verify antigen typing label to unit and place on the blue card. |  |

**D. Gel Testing for Antigen typing using MTS Buffered Gel Card**

 **(Anti-K, -S, other monoclonal)**

Chemical Risk Assessment: Low

Biological Risk Assessment: Low

Protective Equipment: Lab coat, gloves

Reagents:Positive (Heterozygous) Cell and Negative 0.8% Cell for Controls, MTS Diluent 2

 Appropriate MTS Buffered gel card

Supplies: 50 ul, 10 uL pipettes

 Forms: Antigen Typing Worksheet (BB.FORMS.1003), WBFH Antigen Typing Card (donor units)

Equipment Diluent Dispenser, Ortho Workstation

Specimen Requirements:

Anticoagulated blood is preferred. The specimen should be tested as soon as possible after collection.

If delay in testing should occur, the specimen must be stored at 1° to 10°C. Blood drawn into EDTA should not be stored for longer than seven days. It is best to test oxalated or heparinized blood samples within two days of being drawn. Clotted specimens are suitable, but difficulty may be experienced in obtaining the required volume of red blood cells for the procedure. Red blood cells from clots, or from samples collected into ACD or CPD, may be treated up to 21 days after collection.

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **1.0** | **Compare patient identification and required patient information on requisition and blood specimen before proceeding to antigen typing for patients or confirm donor number for donor samples.** *Refer to: Protocol: Comparing Patient Identification Prior to Testing (BB.PROTOCOL.1040)* |  |
| **2.0**  | **Verify that the patient has not been transfused with red blood cells during the previous 3 months.** |  |
| **3.0** | **Check to see if autocontrol or DAT was performed on the specimen.**Note: Red blood cells with a positive DAT cannot be used in the indirect antiglobulin test for antigen typing. |  |
| **4.0** | **Check the rare antisera worksheets to see if the antisera has had quality control performed within the past 24 hours.**4.1 Proceed to testing in step 6.0 if quality control has been performed within the  past 24 hours.* 1. Proceed to next step if quality control has not been performed.
 |  |
| **5.0** | **Select reagent red cells to be used for quality control.**5.1 Select a Positive control which is a cell heterozygous for antigen. a. Example: Positive control for anti-E should be a cell positive for E and e.5.2 Select a Negative control which is a cell negative for antigen. a. Example: Negative control for anti-E should be a cell negative for E. |  |
| **6.0** | **Review the manufacturer’s insert for the antisera being used to confirm procedure.** |  |
| **7.0** | **Visually inspect gel card, then label card with donor unit # or patient identification.** 6.1 Label controls when needed with positive (plus vial number) or negative  control (plus vial number).6.2 Label with antisera being tested. |  |
| **8.0**  | **Remove the foil seal from the MTS Anti-IgG Card. Use within 1 hour of removal.**  |  |
| **9.0** | **Add 25 uL of the antisera to the appropriate reaction chamber of the opened well.** |  |
| **10.0** | **Dispense 50uL of the 0.8% suspension into the appropriate well of the gel card.**10.1 Observe that the contents of the reaction chamber(s) are combined. If necessary tap  GENTLY. |  |
| **11.0** | **Centrifuge gel card(s) in the for 10 minutes.**11.1 After centrifugation remove the gel card(s) and read the front/back for agglutination results. |  |
| **12.0** | **Grade and record results in computer or during downtime record on Antigen Typing Worksheet for donor units or Patient Summary Sheet for patient or Rare Antisera QC if QC was performed.***Refer to: Attachment 2: BB.FORMS.1081 Rare Antisera Quality Control**Refer to: Attachment 3: BB.FORMS.1003 Antigen Typing Worksheet**Refer to: Attachment 4: BB.FORMS.1195 Antibody Identification Summary* |  |
| **13.0** | **Interpretation of Controls if performed.**

|  |  |  |
| --- | --- | --- |
| **Control** | **Acceptable** | **Not Acceptable** |
| **Positive**  | Reaction strength of ≥2+ | Reaction strength < 2+ or Nonreactive |
| **Negative**  | Nonreactive | Reactive any strength |

13.1 Repeat any testing performed and controls if not acceptable.13.2 Report to management any antisera that repeats unacceptable and remove  from use.  |  |
| **14.0** | **Complete a blue WFBH Antigen Typing Card for donor units and place the SCC antigen label that prints out on the blue card .***Refer to Attachment 5: WFBH Antigen Typing Card (Blue)*14.1 Carefully verify antigen typing label to unit and place on the blue card. |  |

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

 All procedures must be reviewed as stated 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: Direct Antiglobulin Procedure

 Specials: Acid Elution/Screen for Thermal Amplitude and Specificity of Cold Autoagglutinins/Adsorption

**5. References**:

 Technical Manual, American Association of Blood Banks (AABB). Revised periodically

 Standards for Blood Banks and Transfusion Services. Revised periodically

Sanford, Kimberly W. and Roseff, Susan D. Detection and Significance of Donath-Landsteiner

Antibodies in a 5-year-old Female Presenting with Hemolytic Anemia. Lab Medicine. April 2010.

Immucor PanoCell8 Direction Circular

Gamma-Quin Chloroquine Diphosphate Solution Direction Circular, 9/2010

Ortho manufacturer’s inserts, Revised periodically.

**6. Attachments/Links**:

***Donath-Landsteiner (DL) Test Worksheet***

***Rare Antisera Quality Control***

***Antigen Typing Worksheet***

***Antibody Identification Summary Sheet***

***WFBH Antigen Typing Card (blue)***

***Reagent Label***

***ABID Charging Flow sheet***

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

 See Document Change Control