|  |  |  |  |
| --- | --- | --- | --- |
|  | **DIRECT ANTIGLOBULIN TEST – Including COLD DAT** | **Dept:**  | 324311 |
| **Dept Name** | Blood Bank |
| **Effective Date:** | Title 21 |
| **Revised Date:** | Title 21 |
| **Name & Title**: CLIA Laboratory Medical Director | **Contact:** | Julie H. Simmons/Christina S. Warren |
| **Signature:** | Title 21 | **Date:** | **Title21** |

**1. General Procedure Statement:**

1. **Purpose:**

**DAT:**

The Direct Antiglobulin Test is used to determine if immunoglobulins (IgG), complement

(C3b and/or C3d) or both are coating red cells. This test does not distinguish between the two. The DAT is used to investigate hemolysis due to hemolytic transfusion reactions, Hemolytic Disease of the fetus and newborn, autoimmue hemolytic anemias, drug induced immune hemolysis and other autoimmune conditions.

Some antibodies are capable of binding complement when they attach to the red cell membrane. The following are examples of antibodies that bind complement most often: Anti-A, -B, -Lea, -Leb, -Jka,

-Jkb, -Sc1, -Co3, -Ge2, -Ge3, -I, -i, -P, -PP1Pk and –Vel. The following antibodies can bind complement but it is rare: Anti-D, -P1, -Lua, -Lub, -Kell, -Fya, -Fyb, -Coa, -Cob, -Dia, -S, -s and -Yta.

If a cold autoantibody is suspected based on lab results and patient symptoms and there is no reaction with anti-C3d, then anti-C3b,-C3d may be used to prove the presence of complement on cells. This can happen in diseases like Paroxysmal Cold Hemoglobinuria. Sometimes only C3b is present so testing with the combination anti-C3b,-C3d reagent will show that complement is indeed on the red cells. Testing with anti-C3b3d is performed at the direction of management or the Medical Director.

 **Cold DAT:**

The Direct Antiglobulin Test (DAT) is used to determine if immunoglobulins (IgG), complement

(C3b and/or C3d) or both are coating red cells. This specialized DAT technique is used to investigate Warm Auto Immune Hemolytic Anemias (WAIHA) that have a negative routine DAT. There are some patients (approximately 2-11%) who exhibit the clinical symptoms and laboratory findings consistent with WAIHA but the routine DAT testing is negative. There are several reasons why this happens:

* Too little IgG on the cells to be picked up with routine testing
* The antibody is IgM or IgA
* The IgG coating the cells has a low affinity for the red cells and dissociates from the red cells under normal testing conditions

The purpose of this test is to determine if low affinity IgG is coating the patient’s red cells.

The use of a cold wash and keeping the cells at cold temperatures has shown to help keep the low affinity anti-IgG antibodies attached to red cells.

**B.** **Responsible Department/Scope:**

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

 ii. Procedure prepared by: Julie Jackson

 iii. Who performs procedure: Department staff/management

**C. Definitions:**

MR#: Medical Record number

PBS: Phosphate Buffered Saline

DAT: Direct Antiglobulin Test

Blood Bank requisition or equivalent: Blood Bank Requisition, antibody ID summary

Check Cells: IgG coated or C3d coated red blood cells used to check the reactivity of anti-IgG or anti-C3d

 reagent when a negative reaction is obtained.

SC: Saline control that is tested as part of the DAT. Saline control should always be negative. False

 positives may occur due to strong cold autoantibodies.

 COLD: temperature between 4-6˚C

 WAIHA: Warm Auto Immune Hemolytic Anemia

 Elu Wash: Working Wash Solution prepared from concentrated Wash

 Solution in the Immucor Gamma ELU-KIT II. Must be kept COLD between 4-6˚C.

 Poly AHG: Anti-IgG,-C3d antisera

**D. Sections**:

 I. Protocols ***Antibody Identification Policies: Direct Antiglobulin Testing (DAT) Protocol; BB.Routine.1008***

II. Polyspecific DAT Tube Tests (Poly AHG, IgG, C3d)

III. IgG Gel Test

IV. C3b,-3d Tube Test

V. Computer Entry

VI. Cold DAT

1. Pretesting Steps
2. Cold DAT Testing: Tube Method
3. Cold DAT Testing: Gel Method

**2. Procedure: II. DAT Tube Tests (Polyspecific AHG, IgG, C3d)**

 Chemical Risk Assessment: Low

Biological Risk Assessment: Low

Protective Equipment: Lab coat, gloves

 Reagents:Polyspecific AHG, Anti-IgG, Anti-C3d

 0.9% saline or PBS

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

 Dispo pipettes

 Equipment: Plasma Prep Centrifuges or equivalent

 Light magnifying lamp

 Serofuge or CW2 centrifuge

 Specimen Requirements: Specimens with gross hemolysis or contamination should not be used. EDTA specimens or donor

 blood stored in citrate anticoagulant can be tested.

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **1.0** | **Label a 12x 75 mm clean test tube with patient last name and MR#.** |  |
| **2.0** | **Prepare a 3-5% suspension of patient red cells with isotonic saline or PBS.**  |  |
| **3.0** | **Label four (4) 10x75mm tube with a minimum of the first three (3) letters of the patient's last name and abbreviations of test.****3.1 Label one of the tubes "DAT", one tube “IgG”, one tube “C3d” and one tube “SC” for** **(Saline Control).** |  |
| **4.0** | **Place one drop of the prepared 3-5% cell suspension into the each labeled tube.**  |  |
| **5.0** | **Wash labeled tubes containing the drop of 3-5% cell suspension a minimum of three (3) times in automatic cell washers OR alternately wash with tubes full of isotonic saline or PBS, decanting completely after the last wash.**  |  |
| **6.0** | **Obtain washed labeled tubes and add 2 drops of appropriate antisera to each of the washed cells tubes. *Refer to chart below.***6.1

|  |  |  |
| --- | --- | --- |
| **Tube Labeled** | **Antisera** |  **Amount** |
| DAT | Polyspecific AHG | 2 drops |
| IgG | Anti-IgG | 2 drops |
| C3d | Anti-C3d | 2 drops |
| SC | Saline | 2 drops |

 |  |
| **7.0** | **Mix tube contents well and centrifuge immediately for 20 seconds at 3300 -3500 rpm or at the calibrated speed for immediate spin room temperature.**  |  |
| **8.0** | **Remove one tube from centrifuge at a time and gently resuspend cells, examining immediately macroscopically using a magnifying lamp and microscopically for agglutination.**8.1 Observe both positive and negative reactions microscopically. 8.2 Check all positive reactions microscopically for mixed field regardless of strength of  reactivity.8.3 NOTE: Agglutination reactions with weakly sensitized erythrocytes may be very  fragile. Extreme care should be taken when resuspending the cell button. |   |
| **9.0** | **Grade reactions and record results on BB requisition or equivalent.*****Refer to Routine: BB.R.1018 Grading of Positive and Negative*** *Reactions* |  |
| **10.0** | **Interpret reactions and determine if further incubation is required.****10.1**

|  |  |  |
| --- | --- | --- |
| **Test** | **Interpretation** | **Instruction** |
| **Pos** | **Neg** | **SC** |
| **DAT IS** | **POS** |  | **NEG** | *Proceed to step 12* |
| **DAT IS** |  | **NEG** | **NEG** | *Proceed to 5 minute incubation, step 11* |
| **DAT IS** | **POS** |  | **POS** | *Invalid due to Positive SC* |
|  |
| **IgG Tube IS** | **POS** |  | **NEG** | *Proceed to step 12* |
| **IgG Tube IS** |  | **NEG** | **NEG** | *Proceed to 5 minute incubation, step 11* |
| **IgG Tube IS** | **POS** |  | **POS** | *Invalid due to Positive SC* |
|  |
| **C3d Tube IS** | **POS** |  | **NEG** | *Proceed to step 12* |
| **C3d Tube IS** |  | **NEG** | **NEG** | *Proceed to 5 minute incubation, step 11* |
| **C3d Tube IS** | **POS** |  | **POS** | *Invalid due to Positive SC* |

 **IS = Immediate Spin** |  |
| **11.0** | **Incubate initially negative tubes for 5 minutes at room temperature (RT) and then**  **repeat steps 7.0, 8.0 and 9.0.***NOTE: Weak anti-complement reactions may be enhanced by incubation at RT*  *following examination of the immediate spin reaction.*11.1 After the 5 minute incubation: a. Add 1 drop of appropriate check cells to each negative tube.

|  |  |  |
| --- | --- | --- |
| Tube Labeled | Check Cells |  Amount |
| DAT | IgG Coated | 1 drop |
| IgG | IgG Coated | 1 drop |
| C3d | C Coated | 1 drop |
| SC | None | NA |

b. Mix well.c. Centrifuge for 20 sec or at the calibrated speed for immediate spin room  temperature.d. Gently resuspend, examining macroscopically for agglutination.e. Grade and record reactionon BB requisition or equivalent. (Reaction strength must be at least 2+ for IgG coated cells. If reaction strength < 2+, test must be  repeated, except Saline control tube should be negative.)f. Consult management if Saline control (SC) tube is positive before reporting results. g. Proceed to step 12.0 |  |
| **12.0** | **Determine if an elution is required after interpretation of DAT tests.** ***Refer to (Routine): Protocol, Direct Antiglobulin Testing (DAT)*** |  |
| **13.0** | **Computer entry**13.1 Refer to Section V: Computer Entry.13.2 Record on Blood Bank requisition or equivalent with the other DAT results. |  |

**2. Procedure: III. IgG Gel Test**

 Chemical Risk Assessment: Low

 Biological Risk Assessment: Low

 Protective Equipment: Lab coat, gloves

Reagents:IgG Gel card

 MTS Diluent 2

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

 Pipets to dispense 10uL and 50uL

 Equipment: Ortho Workstation

 Specimen Requirements: Specimens with gross hemolysis or contamination should not be used. EDTA specimens or donor

 blood stored in citrate anticoagulant can be tested. Recommended that testing be completed within

 24 hours of collection.

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **1.0** | **Label a clean 12x75 mm test tube with patient last name and MR#.** |  |
| **2.0** | **Retrieve IgG gel card and MTS Diluent 2 and bring to room temperature.**  |  |
| **3.0** | **Prepare a 0.8% suspension of patient red cells.** 3.1 Pipet 1 ml MTS Diluent 2 into labeled test tube.3.2 Add 10 ul packed red blood cells into the diluent.3.3 Mix gently. |  |
| **4.0** | **Label a micro IgG gel card with a minimum of the first 3 letters of the patient's last name and "IgG".**  |  |
| **5.0** | **Remove the foil seal from the anti IgG card.**5.1 Visually inspect the IgG gel card to ensure that residual film does not block the opening  of any microtube. 5.2 Check gel card for liquid layer on top of gel, make sure gel is not  dried out and no trapped bubbles in gel or artifacts. 1. If there are liquid bubbles in the upper chamber, then centrifuge the card in the DG spin before using.

5.3 Foil should be removed immediately before testing or within 1 hour of testing. a. Once opened, the gel may begin to dry out. |  |
| **6.0** | **Add 50 ul of prepared 1.0% patient cell suspension into the microtube IgG gel card using an appropriate MLA pipette ensuring there is an air gap in the microtube.**6.1 The test must be repeated if the air gap is not present.Air Gap No Air Gap |  |
| **7.0** | **Centrifuge for 10 minutes in the Ortho workstation centrifuge.**  |  |
| **8.0** | **Remove the gel card from centrifuge. and read front of gel tubes against a white background or held up to light for agglutination and/or hemolysis.**  |   |
| **9.0** | **Read front/back of gel tubes against a white background or held up to light for agglutination and/or hemolysis.** ***Refer to Routine: BB.R.1018 Grading of Positive and Negative Reactions***

|  |  |
| --- | --- |
| **Expected results** | **Definition** |
| **Positive** | Agglutination and/or hemolysis of the red blood cells is a positive test result. Red blood cells may remain suspended on the top of the gel or be dispersed throughout the gel in varying degrees. A few red blood cells may form a button in the bottom of the microtube in some positive reactions. |
| **Negative** | No agglutination and no hemolysis of the red blood cells is a negative test result. A complete sedimentation of all red cells is present in the bottom of the microtube. |

 |  |
| **10.0** | **Record the results in SCC Computer and on requisition and Antibody Summary form as applicable.**10.1 Refer to Section V: Computer Entry.10.2 Requisition/Antibody Summary form Record on Blood Bank requisition or equivalent with the other DAT results. |  |
| **11.0** | **Determine if elution is needed.** ***Refer to Routine: BB.R.1008: Protocol, Direct Antiglobulin Testing (DAT)******Refer to Specials: BB.SP.1003: Acid Elution*** *11.1 If only the IgG Gel DAT is positive, then the eluate should be tested in gel for 40 minutes.*  |  |

**2. Procedure: IV. C3b,-3d Test**

Chemical Risk Assessment: None

Biological Risk Assessment: None

Protective Equipment: Lab coat, gloves

Reagents:Anti-C3bC3d

 0.9% saline or PBS

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

 Dispo pipettes

 Equipment: Plasma Prep Centrifuges or equivalent

 Light magnifying lamp

 Serofuge or CW2 centrifuge

 Specimen Requirements: Specimens with gross hemolysis or contamination should not be used. EDTA specimens or donor

 blood stored in citrate anticoagulant can be tested.

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **1.0** | **Label a clean 12x75 mm test tube with patient last name and MR#.** |  |
| **2.0** | **Prepare a 3-5% suspension of patient red cell with isotonic saline or PBS.**  |  |
| **3.0** | **Label a 10x75mm tube with a minimum of the first three (3) letters of the patient's last name and "C3bd".**  |  |
| **4.0** | **Place one (1) drop of the 3-5% patient cell suspension into the tube labeled C3bd.**  |  |
| **5.0** | **Wash cells a minimum of three (3) times in automatic cell washers OR alternately wash with tubes full of isotonic saline or buffered PBS , decanting completely after the last wash.**  |  |
| **6.0** | **Immediately add the appropriate number of drops of anti-human C3bd depending on the manufacturer used to the washed cells.**6.1 For Immucor anti-C3b,-C3d, use one or two (1-2) drops.  |  |
| **7.0** | **Mix tube contents well.**  |  |
| **8.0** | **Incubate for 5 minutes at room temperature.** |  |
| **9.0** | **Centrifuge for 15-20 seconds (depending on the centrifuge’s calibrated seconds for room temperature) at 3400 rpm or at the calibrated speed for room temperature.** |   |
| **10.0** | **Remove tubes from centrifuge.** 10.1 Gently resuspend cells, examining immediately macroscopically using a magnifying  lamp and microscopically for agglutination without delay.10.2 Observe all positive reactions for mixed field regardless of strength of reactivity.*NOTE: Agglutination reactions with weakly sensitized erythrocytes may be very fragile. Extreme care should be taken when resuspending the cell button.* |  |
| **11.0** | **Read and grade reactions.** ***Refer to Routine: BB.R.1018 Grading of Positive and Negative Reactions*** |  |
| **12.0** | **Record results in SCC and on BB requisition or Antibody Identification Summary sheet and determine next steps.** |  |
| **If** | **Then** |
| **NEGATIVE** | 1. Add 1 drop of **complement** control cells to the tube, mix well.
2. Centrifuge for 20 sec or at the calibrated speed for room temperature.
3. Gently resuspend, examining macroscopically for agglutination.
4. Grade and record reactionon BB requisition or equivalent.

Complement control cells expected reactions: w+ - 4+ If Complement control cells: neg - micro +, repeat testIf Complement control cells: w+-4+, proceed1. Proceed to step 13.0
 |
| **POSITIVE**  | Proceed to step 13.0 |
|  |
| **13.0** | **Determine if elution is needed.** ***Refer to Routine: BB.R.1008: Protocol, Direct Antiglobulin Testing (DAT)******Refer to Specials: BB.SP.1003: Acid Elution***

|  |  |
| --- | --- |
| **IF** | **Then** |
| **NEGATIVE** | No further action unless directed by management |
| **POSITIVE** | Perform Elution(*see Special Procedures; Acid Elution; BB.SP.1003)* |

 |  |

**2. Procedure: V. Computer Entry**

Chemical Risk Assessment: None

Biological Risk Assessment: None

Protective Equipment: Lab coat, gloves

Reagents:NA

 Supplies: NA

 Equipment: Computer

 Specimen Requirements: NA

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **1.0** | **Add DAT in SCC by going to Patient>Orders>Modify if patient has a current Blood Bank order.**1.1 Order testing in Beaker if no current order.  |  |
| **2.0** | **Select either DATX for complete profile or individual DAT method from drop down under Tests.**1. F12 to Accept.
 |  |
| **3.0** | **Enter Results in SCC either by worksheet or individually.** **3.1**

|  |  |
| --- | --- |
| **Using Worksheet** | **Individually** |
| 1. Go to Results>Patient Test Worksheets
 | 1. Go to Patient>Orders>Results.
 |
| 1. Build>DAT Profile
2. F12 to accept.
 | 1. Enter patient’s MRN.
2. F12 to accept.
 |
| 1. Select Tests.
2. F12 to accept.
 | 1. Select correct order and enter.
 |
| 1. Go To Results>Enter\_results
 | 1. Select correct test by double clicking on test to result.
 |
| 1. Enter results and F12 to accept.
 | 1. Enter results and F12 to accept.
 |

 |  |
| **4.0** | **Add Elution Test to DATX accession number when elution is performed.**4.1 Go to Patient>Orders>Modify.4.2 Enter Patient’s MRN and F12 to accept.4.3 Select Elution from drop down Tests.4.4 F12 to accept**.**  |  |

**2. Procedure: VI: Cold DAT**

Chemical Risk Assessment: None

Biological Risk Assessment: None

Protective Equipment: Lab coat, gloves

Supplies: 10x75 or 12x75 test tubes

Transfer pipets

Ice Bath (4-6˚C)

MLA and ID-Tipmaster pipettes

Blood Bank requisition or equivalent

Reagents: Anti-IgG,-C3d (poly AHG) antisera

Anti-IgG antisera

Anti-C3b,-C3d antisera

IgG sensitized control cells

Complement control cells

Saline (0.85%)

 Anti-IgG gel card

 Neutral Gel card

MTS Diluent 2

Equipment: Agglutination lamp

Microscope

Specimen centrifuge: Plasma Prep centrifuge or EBA 20 centrifuge

Testing centrifuge: Sero-fuge

Ortho Workstation

Specimen Requirements:

A properly labeled EDTA tube:

6.0mL pink top is preferred.

3.0mL or 10.0mL lavender top EDTA is also acceptable

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

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

**Section A: Pretesting Steps**

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **1.0** | **The following reagents and supplies need to be COLD (4-6˚C) at time of use:****Keep refrigerated or in ice bath until ready to use.**

|  |  |  |
| --- | --- | --- |
| **For Tube Testing- Part A** | **For Gel Testing- Part B** | **For *both*** **Tube and Gel Testing** |
| **0.85% Saline** Place 3-4 drops in a tube and place in COLD ice bath or refrigerator for a minimum of 10 minutes before use | **Gel card – IgG****Gel card – Neutral (Buffered saline)**Place in the refrigerator for a minimum of 10 minutes before use | **EluWash** Remove koozie if placing in ice bath |
| **Poly AHG** Use a COLD vial from the refrigerator | **MTS diluent** 1. A clean 12x75mm tube with 1.0 mL of MTS Diluent 2.
2. An extra tube with a few extra drops in case needed.

Place both in the refrigerator for a minimum of 10 minutes before use |
| **Anti-IgG** Use a COLD vial from the refrigerator |
| **Anti-C3b,-C3d** Use a COLD vial from the refrigerator |

Note: Check cells do not need to be COLD**If using an ice bath have a towel or paper towels handy for drips.** |  |
| **2.0** | **Proceed to Testing****2.1 Go to Section II for Cold DAT testing: Tube Method****2.2 Go to Section III for Cold DAT testing: Gel Method** |  |

**Section B: Cold DAT Testing: Tube Method**

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **1.0** | **Label four 10x75mm tubes with a minimum of the first 3 letters of the patient's last name and test abbreviation.**

|  |  |
| --- | --- |
| **Test tube** | **Abbreviation** |
| Poly AHG tube | DAT |
| Anti-IgG tube | IgG |
| Anti-C3b,-C3d tube | C3 |
| Saline Control tube | SC |
|  |  |

**1.1 Test abbreviations:**  |  |
| **2.0** | **Prepare a 3-5% cell suspension of the patient’s red cells with isotonic saline or PBS:**2.1 Label a clean 12x 75 mm test tube with patient last name and MR#. 2.2 Add 1-2 drops of packed patient red cells into the properly labeled tube.2.3 Add 0.85% saline to produce a 3% to 5% red cell suspension.2.4 Mix red cell suspension.2.5 Visually compare color of suspension with that of a 3-5% commercial reagent red cell suspension.1. If it appears <3%, add patient red cells to achieve a 3-5% suspension.
2. If it appears >5%, add saline to suspension to achieve a 3-5% suspension.
 |  |
| **3.0** | **Place one drop of the 3-5% patient cell suspension into each labeled 10x75mm tube from step 1.0.**  |  |
| **4.0** | **Chill tubes to 4-6˚C in refrigerator or ice bath at bench.** **4.1 Chill for at least 10 minutes.** |  |
| **5.0** | **Wash cells a minimum of 3 times with tubes full of COLD Elu Wash, decanting completely after the last wash.** 5.1 Keep the Elu Wash COLD at all times (in refrigerator or ice bath at bench)5.2 After washing tubes keep COLD at all times(in refrigerator or ice bath at bench) |  |

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **6.0** | **Add COLD anti-sera or saline to appropriately labeled washed cells:****6.1 Do Coombs testing:** |  |
|  | **To tube labeled:** | **Add:** | **Instructions:** |  |
| DAT | 2 drops of Poly AHG | Proceed to step 7.0 |
| IgG | 2 drops of Anti-IgG | Proceed to step 7.0 |
| SC | 2 drops of Saline | Proceed to step 7.0 |
| C3 | 2 drops of Anti-C3b,-C3d | Proceed to step 11.0 (Do not do Immediate spin) |
|  |
| **7.0** | **Mix tube contents well and centrifuge immediately for the calibrated time for immediate spin room temperature at 3400-3600 rpm.**  |  |
| **8.0** | **Remove tubes from centrifuge and read.** 8.1 Gently resuspend cells, examining immediately for agglutination 1. Read macroscopically using a magnifying lamp
2. If negative macroscopically then examine microscopically.

8.2 Observe all positive reactions for mixed field regardless of strength of reactivity.8.3 Immediately place back in refrigerator or ice bath**NOTE:** Agglutination reactions with weakly sensitized erythrocytes may be very  fragile. Extreme care should be taken when resuspending the cell button. |   |
| **9.0** | **Grade and record results on Antibody Identification Summary or equivalent. Be sure to record as being a Cold DAT.** |  |
| **10.0** | **After immediate spin:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Tube label:** | **If:** | **Result is:** | **Then** |
| DAT | Poly AHG | Negative | proceed to step 11.0 |
| Positive | proceed to step 17.0 |
| IgG | Anti-IgG | Negative | proceed to step 11.0 |
| Positive | proceed to step 17.0  |
| SC | Saline control | Negative | proceed to step 11.0 |
| Positive | Results of all DATs are invalid |
|  |  |  |  |

 |  |
| **11.0** | **Incubate for 5 minutes at 4-6˚C.** 1. Incubate in refrigerator or ice bath at bench
 |  |
| **12.0** | **Mix tube contents well and centrifuge immediately for the calibrated time for immediate spin room temperature at 3400-3600 rpm.**  |  |
| **13.0** | **Remove tubes from centrifuge and read.** 13.1 Gently resuspend cells, examining immediately for agglutination 1. Read macroscopically using a magnifying lamp
2. If negative macroscopically then examine microscopically.

13.2 Observe all positive reactions for mixed field regardless of strength of reactivity.13.3 Immediately place back in refrigerator or ice bath**NOTE:** Agglutination reactions with weakly sensitized erythrocytes may be very  fragile. Extreme care should be taken when resuspending the cell button. |   |
| **14.0** | **Grade and record results on Antibody Identification Summary sheet or equivalent. Be sure to record as being a Cold DAT.** |  |
| **15.0** | **After reading at 5 minutes incubation at 4-6˚C:**

|  |  |  |  |
| --- | --- | --- | --- |
| **Tube label:** | **If:** | **Result is:** | **Then** |
| DAT | Poly AHG | Negative | proceed to step 16.0 |
| Positive | proceed to step 17.0 |
| IgG | Anti-IgG | Negative | proceed to step 16.0 |
| Positive | proceed to step 17.0  |
| C3 | Anti-C3b,-C3d | Negative | proceed to step 16.0 |
| Positive | proceed to step 17.0 |
| SC | Saline control | Negative | proceed to step 16.1.d |
| Positive | Results of all DATs are invalid |
|  |  |  |  |

 |  |

|  |  |  |
| --- | --- | --- |
| **STEPS** | **INSTRUCTIONS** | CHANGE/APPROVAL |
| **16.0** | **After the 5 minute incubation:** 16.1 Add 1 drop of check cells to the tube, mix well. 1. Check cells do NOT need to be COLD
2. Use Coombs check cells for Poly and IgG tubes
3. Use Complement check cells for C3d tube
4. None are needed for the Saline Control
* The saline control must be negative for DAT results to be valid

16.2 Centrifuge at the calibrated time for immediate spin room temperature.16.3 Gently resuspend, examining macroscopically for agglutination.16.4 Grade and record reactionon Antibody Identification Summary or equivalent.1. Reaction strength must be at least 2+.
2. If reaction strength < 2+, test must be repeated.
 |  |
| **17.0** | **DAT Interpretation*****Go to (Routine): Antibody Identification Policies:***  ***Direct Antiglobulin Testing (DAT) Protocol; BB.Routine.1008*** |  |
| **If** | **Then** |
| **NEGATIVE** | No further actions unless directed by management.  |
| **POSITIVE** | Perform Elution if not already done. ***see Special Procedures; Acid Elution; BB.SP.1003*** |
|  |
| **18.0** | **Computer entry**18.1 Computer entryCOLD DATs are not entered in the computer at this time.18.2 Record on Antibody Identification Summary sheet or equivalent with the other DAT results. Make sure to note that testing was done at 4-6˚C/COLD. |  |
| **19.0** | **Confirm with management and send sample to Blood Center of Wisconsin for DAT Negative Hemolytic Anemia Evaluation.** 19.1 Obtain sample collected from same approximate time or request additional sample if  patient has not been transfused. a. Recommended sample: Two 10ml Clot tubes (Red top) and 5ml EDTA Whole  blood (pink or lavender) |  |

**Section C: Cold DAT Testing: Gel Method**

| **STEPS** | **INSTRUCTIONS** | **CHANGE/****APPROVAL** |
| --- | --- | --- |
| **1.0** | **Label the COLD gel IgG card and COLD neutral gel (buffered saline) card from Section I step 1.0 with patient last name and MR#.**1.1 Keep gel cards COLD until ready to spin. |  |
| **2.0** | **Label the COLD 12 x 75 mm test tube with 1.0 ml of MTS Diluent 2 from Section I step 1.0****with patient last name, MR# and “1.0%”.** |  |
| **3.0** | **Label a 12x75mm tube labeled with patient last name, MR# and “Gel”.** 3.1 Add 3-5 drops of packed cells to tube and place in refrigerator or ice bath on bench to chill to 4-6˚C for at least 10 minutes. |  |
| **4.0** | **Wash cells a minimum of 3 times with tubes full of COLD Elu Wash, decanting completely after the last wash.**  ***Note:*** *Remove EluWash with pipette to reserve as many red cells as possible.*4.1 Keep the Elu Wash cold at all times (in refrigerator or ice bath on bench)4.2 After washing keep tubes cold at all times(in refrigerator or ice bath on bench) |  |
| **5.0** | **Prepare a 1.0% suspension of washed patient red cells with COLD MTS Diluent 2:**5.1 Add 10 ul of COLD washed packed red cells into the COLD diluent tube from step 2.0.5.2 Mix gently.5.3 Keep tube cold at all times(in refrigerator or ice bath on bench) |  |
| **6.0** | **Remove the foil seal from the COLD anti IgG card and COLD Buffered Saline gel card.**6.1 Visually inspect the cards to ensure that residual film does not block the opening  of any microtube. Check gel cards for liquid layer on top of gel, make sure gel is not  dried out.6.2 Foil should be removed immediately before testing or within 1 hour of testing. Once opened, the gel may begin to dry out. |  |
| **7.0** | **Add 50 ul of 1.0 % COLD, washed patient’s cell suspension into the microtube IgG gel card and 50ul of 1.0 % COLD, washed patient’s cell suspension into the microtube gel buffered saline card.**7.1 Ensure there is an air gap in the microtube. If no air gap, then the test must be  repeated. Air Gap No Air Gap7.2 The gel buffered saline card serves as a control for the procedure.  |  |
| **8.0** | **Immediately centrifuge for 10 minutes in the Ortho workstation centrifuge.**  |  |
| **9.0** | **Remove the gel cards from centrifuge and read front and back of card for each microtube macroscopically for agglutination and/or hemolysis.** |   |
| **10.0** | **Grade and record results on Antibody Identification Summary form or equivalent. Be sure to record results as being a Cold DAT and indicate IgG gel card or buffered saline (control) card.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Expected Results –****IgG gel** | **Expected Results – buffered** | **Interpretation of Results**  | **Definition**  |
| **Positive** | **Negative** | Positive  | Agglutination and/or hemolysis of the red blood cells is a positive test result. Red blood cells may remain suspended on the top of the gel or be dispersed throughout the gel in varying degrees. A few red blood cells may form a button in the bottom of the microtube in some positive reactions. Refer to Routine: Grading of Positive and Negative Reactions. |
| **Negative** | **Negative** | Negative | No agglutination and no hemolysis of the red blood cells is a negative test result. A complete sedimentation of all red cells is present in the bottom of the microtube. |
| **Positive/****Negative** | **Positive** | Invalid  | Buffered gel card is positive and may indicate interference due to a cold autoantibody or some other interfering substance.  |

 |  |
| **11.0** | **DAT Interpretation** |  |
| **If** | **Then** |
| **NEGATIVE** | No further testing |
| **POSITIVE** | Perform Elution if it has not already been done.  ***see Special Procedures; Acid Elution; BB.SP.1003*** |
|  |
| **12.0** | **Computer entry**12.1 Computer entryCOLD DATs are not entered into the computer at this time.12.2 Record on Antibody Identification Summary form or equivalent with the other DAT results. Make sure to note that testing was done at 4˚-6˚C/COLD. |  |
| **13.0** | **Check with management and send sample to Blood Center of Wisconsin for DAT Negative Hemolytic Anemia Evaluation.** 13.1 Obtain sample collected from same approximate time or request additional sample if  patient has not been transfused. a. Recommended sample: Two 10ml Clot tubes (Red top) and 5ml EDTA Whole  blood (pink or lavender) |  |

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

 All procedures must be reviewed as stated in the Document Change 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:**

Special Procedures; Acid Elution; BB.SP.1003

**5. References**:

Immucor package insert. Revised periodically.

Ortho package inserts. Revised periodically.

AABB Technical Manual. Revised periodically.

**6. Attachments**:

NA

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

 Refer to archive history/Title21