TRAINING UPDATE

Lab Location:	SGAH and WAH	**Date Implemented:**	11.30.2012
Department:	Blood Bank	**Due Date:**	12.28.2012

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:
Direct Antiglobulin Test (DAT)
Description of change(s):
• Omitted requirement to test weak positive reactions with check cells

EMPLOYEE SIGNATURES

I have read and understand the procedure described above:

Name	**Signature**	**Date**

Technical SOP

Title	**Direct Antiglobulin Test (DAT)**	
Prepared by	Stephanie Codina	Date: 1/16/2010
Owner	Stephanie Codina	Date: 1/16/2010

Laboratory Approval	**Local Effective Date:**	
Print Name and Title	**Signature**	**Date**
Refer to the electronic signature page for approval and approval dates.		
Local Issue Date:	Local Effective Date:	

Annual Review		
Print Name	**Signature**	**Date**

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Order Code	Local Code
Direct Antiglobulin Test	Tube test	DAT	N/A

Synonyms/Abbreviations
DAT, Direct Coombs

Department
Blood Bank

2. ANALYTICAL PRINCIPLE

The direct antiglobulin test (DAT) demonstrates in vivo coating of red cells with globulins, particularly IgG and C3. Washed red cells from a patient are tested directly with anti-AHG (polyspecific). If the test is positive, the red cells are subsequently tested with anti-IgG and Anti-C3.

3. SPECIMEN REQUIREMENTS

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	N/A
Specimen Collection and/or Timing	N/A
Special Collection Procedures	N/A
Labeling	Refer to the policy 'Sample Specifications for Blood Bank Testing' for labeling requirements.

3.2 Specimen Type & Handling

Criteria	
Type -Preferred **-Other Acceptable**	Whole Blood (EDTA) Whole Blood (oxalated, citrated) or clotted whole blood
Collection Container	Lavender top tube, dark green top tube, or red top tube (without serum separator)
Volume - Optimum **- Minimum**	10ml 2ml
Transport Container and Temperature	Same as above, at room temperature
Stability & Storage Requirements	Room Temperature: 24 hours
	Refrigerated: EDTA samples <10 days
	Frozen: Unacceptable
Timing Considerations	Test as soon as possible following collection
Unacceptable Specimens & Actions to Take	Frozen, Incomplete or incorrect labeling – refer to procedure 'Sample Specifications for Blood Bank Testing' for details.
Compromising Physical Characteristics	Refer to section 14.
Other Considerations	None

4. REAGENTS

Refer to the Material Safety Data Sheet (MSDS) supplied with the reagents for complete safety hazards. Refer to the section in this procedure covering "SAFETY" for additional information.

4.1 Reagent Summary

Reagents / Kits	Supplier & Catalog Number
Anti-AHG (polyspecific)	Immucor, Cat.# 401010 or equivalent
Anti-IgG	Immucor, Cat.# 409210 or equivalent
Anti-C3b,-C3d	Immucor, Cat.# 4068 or equivalent

4.2 Reagent Preparation and Storage

NOTES: Date and initial all reagents upon opening. Each container must be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4) expiration date, (5) initials of tech, (6) any special storage instructions; check for visible signs of degradation.
Refer to the Material Safety Data Sheet (MSDS) for a complete description of hazards. If a specific hazard is present, it will be noted in this procedure when the hazard is first encountered in a procedural step.

Reagent	Anti-AHG (polyspecific), Anti-IgG, Anti-C3b,-C3d
Container	10ml
Storage	1-10°C
Stability	Stable until manufacturer's expiration date.
Preparation	Ready to use as supplied.

5. CALIBRATORS/STANDARDS

N/A

6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalog Number
IgG coated Control Cells	Immucor, Cat.# 2225 or equivalent
Complement Control Cells	Immucor, Cat.# 7930 or equivalent

6.2 Control Preparation and Storage

NOTE: Date and initial all controls upon opening. Each container should be labeled with (1) substance name, (2) lot number, (3) date of preparation, (4)

expiration date, (5) initials of tech, and (6) any special storage instructions; check for visible signs of degradation.

Control	Coombs Control Cells, Complement Control Cells
Preparation	Resuspend red cells before use by gently inverting each vial several times.
Storage/Stability	1-10°C / Stable until manufacturer's expiration date.

6.3 Frequency

With each negative result.

6.4 Tolerance Limits

Refer to 10.5

6.5 Review Patient Data

N/A

6.6 Documentation

N/A

6.5 Quality Assurance Program

Participation in CAP proficiency testing.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

N/A

7.2 Equipment

Serological centrifuge
Automatic cell washer
Agglutination viewer

7.3 Supplies

12 x 75 mm test tubes and rack
Transfer pipettes
Saline, 0.9%

8. PROCEDURE

NOTE: For all procedures involving specimens, buttoned lab coats, gloves, and face protection are required minimum personal protective equipment. Report all accidents to your supervisor.

The package insert for a new lot of kits must be reviewed for any changes before the kit is used. A current Package Insert is included in the appropriate notebook/file.

Note: This procedure should not be used to test cord blood or heel stick specimens. Refer to procedure "Cord Blood/Heel Stick Routine Testing."

8.1 Polyspecific DAT

Step	Action
1	Confirm specimen acceptability and specimen labeling per procedure, "Sample Specifications for Blood Bank Testing."
2	Label two tubes with the patient identifiers. Labeling standards are detailed in the policy "Sample Specifications for Blood Bank Testing."
3	Label one of the tubes with "AHG."
4	Prepare a 2-4% suspension of patient cells in isotonic saline in the remaining tube per procedure, "Preparing a 2-4% Cell Suspension for Testing." Retain the cell suspension for monospecific DAT testing if indicated.
5	Place 1 drop of cell suspension into the tube labeled "AHG."
6	Wash the tube 3-4 times in saline. Use of an automated cell washer is preferred.
7	Add two drops of polyspecific AHG reagent to the tube labeled "AHG" and mix gently.
8	Serofuge for the saline phase calibration time. Serofugation must take place immediately. If the test system is not serofuged within 1 minute of adding the reagent, the results are considered invalid and test must be repeated.
9	Access the patient information data entry screen using Sunquest function "Blood Order Processing" or utilize a computer downtime form.
10	Remove the tube from the serofuge and verify the labeling of the tubes matches the patient information in the computer or on the downtime form.

Step	Action
11	Gently resuspend the cell button and read macroscopically for agglutination using an agglutination viewer. Immediately record results in the computer or on a downtime form. DATs being performed to rule out suspected transfusion reaction should be read microscopically also. If the tube is A. Positive for agglutination, interpret the test as positive and proceed to Monospecific DATs below. B. Negative for agglutination, incubate at room temperature for up to 5 minutes. **Note**: DO NOT omit immediate spin reading in place of the 5 minute reading. Reactions to IgG coating may become weaker or absent during incubation causing false-negative test results.
12	Serofuge for the saline phase calibration time.
13	Gently resuspend the cell button and read macroscopically for agglutination using an agglutination viewer. Immediately record results in the computer or on a downtime form. DATs being performed to rule out suspected transfusion reaction should be read microscopically also. If the tube is A. Positive for agglutination, interpret the test as positive and proceed to Monospecific DATs below. B. Negative for agglutination, add 1 drop of Coombs Control Cells and gently mix.
14	Serofuge for the saline phase calibration time.
15	Gently resuspend the cell button and read macroscopically for hemolysis and/or agglutination using an agglutination viewer. Immediately record results in the computer or on a downtime form. If the tube is A. Positive for agglutination at a strength $\geq$2+ after the addition of Coombs Control Cells, interpret the polyspecific DAT as negative. No further testing is indicated. B. Negative for agglutination or positive for agglutination at a strength <2+ after the addition of Coombs Control Cells, the results are invalid. Repeat the test.

8.2 Monospecific DATs

Step	Action
1	Label two tubes with the patient identifiers. Labeling standards are detailed in the policy "Sample Specifications for Blood Bank Testing."
2	Label one of tube with each of the following: A. "IGG" B. "C3"
3	Place 1 drop of cell suspension prepared in step 4 above into each labeled tube.
4	Wash the tubes 3-4 times in saline. Use of an automated cell washer is preferred.
5	Add two drops of AHG reagent to each appropriately labeled tube and mix gently. A. Add 2 drops of Anti-IgG to the tube labeled "IGG." B. Add 2 drops of Anti-C3d,C3b to the tube labeled "C3."
6	Serofuge for the saline phase calibration time. Serofugation must take place immediately. If the test system is not serofuged within 1 minute of adding the reagent, the results are considered invalid and test must be repeated.
7	Access the patient information data entry screen in Sunquest using function "Blood Order Processing" or utilize a computer downtime form. Add the IgG and C3 DAT tests as outlined in the LIS section of this procedure.
8	Remove the tubes from the serofuge and gently resuspend the cell button and read macroscopically for agglutination using an agglutination viewer. Immediately record results in the computer or on a downtime form.

If the IgG tube is....	Then...
Positive for Agglutination	Report the result as positive. No further testing is necessary.
Negative for Agglutination	1. Add 1 drop of Coombs Control Cells and gently mix. 2. Serofuge for the saline phase calibration time. 3. Gently resuspend the cell button and read macroscopically for hemolysis and/or agglutination using an agglutination viewer. Immediately record results in the computer or on a downtime form. 4. If the tube is a. Positive for agglutination at a strength ≥2+ after addition of Coombs Control cells, interpret test as negative. No further testing is indicated. b. Negative or positive for agglutination at strength <2+ after the addition of Coombs Control cells, the test is invalid. Repeat.

Step	Action

If the C3 tube is...	Then...
Positive for agglutination	Report the result as positive. No further testing is necessary.
Negative for agglutination	1. Incubate at room temperature for up to 5 minutes. 2. Serofuge for the saline phase calibration time. 3. Gently resuspend the cell button and read macroscopically for agglutination using an agglutination viewer. Immediately record results in the computer or on a downtime form. DATs being performed to rule out suspected transfusion reaction should be read microscopically also. 4. If the tube is a. Positive for agglutination, interpret the test as positive. b. Negative for agglutination, 1) Add 1 drop of Complement Control Cells and gently mix. 2) Serofuge for the saline phase calibration time. 3) Gently resuspend the cell button and read macroscopically for agglutination using an agglutination viewer. Immediately record results in the computer or on a downtime form. 4) If the tube is i. Positive for agglutination at strengths ≥2+ after the addition of Complement Control Cells, interpret the polyspecific DAT as negative. No further testing is indicated. ii. Negative for agglutination or positive for agglutination at strength <2+ after the addition of Complement Control Cells, the results are invalid. Repeat the test.

8.3 Determination of Need for Eluate

Eluate Needed When Any of the Following Is True	No Further Testing Needed When Any of the Following Are True
1. Patient transfused or pregnant within the previous 90 days 2. Patient received out-of-group platelets within the previous 30 days 3. DAT was performed as part of a transfusion reaction workup 4. Patient has no previous history of positive DAT or eluate workup.	1. Negative IgG DAT regardless of polyspecific and anti-C3 DAT results 2. Patient with previous history of positive DAT and/or eluate workup who does not meet any of the criteria listed in column 1

9. CALCULATIONS

N/A

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

If there is....	Then the DAT is interpreted as....
No agglutination in the polyspecific AHG test **and** Coombs Control cells react at strength ≥2+	Negative
Any amount of agglutination in the polyspecific AHG test before or after the 5-minute incubation	Positive with polyspecific AHG. Test with monospecific antisera (anti-IgG and anti-C3d,C3b)
No agglutination with anti-IgG **and** Coombs Control cells react at strength ≥2+	Negative with anti-IgG
Any amount of agglutination with anti-IgG	Positive with anti-IgG
No agglutination with ant-C3d,C3b **and** Complement Control cells react at strength ≥2+	Negative with anti-C3d,C3b
Any amount of agglutination with anti-C3d,C3b before or after the 5-minute incubation	Positive with anti-C3d,C3b

10.2 Rounding

N/A

10.3 Units of Measure

N/A

10.4 Clinically Reportable Range (CRR)

N/A

10.5 Repeat Criteria and Resulting

Reactivity with check cells must be 2+ or greater. If no agglutination is observed or the reactivity is less than 2+, the test is invalid and must be repeated.

11. EXPECTED VALUES

11.1 Reference Ranges

N/A

11.2 Critical Values

None established

11.3 Priority 3 Limit(s)

None established

12. CLINICAL SIGNIFICANCE

N/A

13. PROCEDURE NOTES

- **FDA Status:** Approved/cleared
- **Validated Test Modifications:** None

14. LIMITATIONS OF METHOD

A. The sensitivity of antiglobulin tests is greatly impaired if human protein is introduced into the test system after washing the red blood cells (even when the amount is very small).
B. It is important to centrifuge the test without delay after adding the antiglobulin to the test cells. Progressively diminishing agglutination may accompany delayed centrifugation. If centrifugation is postponed beyond 1 minute, the test is invalid and must be repeated, even if the Coombs control cells yield a positive result.
C. Positive Coombs Control Cells does not provide absolute assurance that false results will not occur.

15. SAFETY

You, the employee, have direct responsibility to avoid injury and illness at work. Nearly all harmful exposures to infectious substances and chemicals, and other injuries, can be avoided with effective training and consistent safe work practices.

Become familiar with the Environmental, Health and Safety (EHS) Manual to the learn requirements on working safely and protecting the environment from harm. Although lab work typically focuses on the hazards of working with specimens and chemicals, we must also control other important hazards.

- Slips, trips, and falls cause many serious injuries. Please ensure that spills are cleaned quickly (to avoid slippery floors) and that you can see and avoid obstacles in your path.
- Ergonomic injuries result from performing tasks with too much repetition, force, or awkward position. Ergonomic injuries include strains and back injuries. Learn about ergonomic hazards and how to prevent this type of injury.
- Scratches, lacerations, and needlesticks can result in serious health consequences. Attempt to find ways to eliminate your risk when working with sharp materials.
- Warnings of other specific hazards are noted in this procedure. Please comply with the requirements to reduce your risk of injury."

Report all accidents and injuries to your supervisor or the Environmental, Health and Safety Coordinator.

16. RELATED DOCUMENTS

SOP: Sample Specifications for Blood Bank Testing
SOP: Acid Elution

17. REFERENCES

1. Roback, J.D., Combs, M.R., Grossman, B.J., Hillyer, C.D. 2008. Technical Manual of the AABB, 16th ed. AABB Publishing, Bethesda, Maryland.
2. Package Insert for Anti-IgG, -C3d; Polyspecific (Murine Monoclonal), ImmucorGamma, Inc., Norcross, GA, Insert Code 3002-2, Revision Date 9/2010.
3. Package Insert for Anti-IgG (Murine Monoclonal), ImmucorGamma, Inc., Norcross, GA, Insert Code 3001-2, Revision Date 09/2010.
4. Package Insert for Anti-C3b,-C3d (Murine Monoclonal), ImmucorGamma, Inc., Norcross, GA, Insert Code 3000-2, Revision Date 09/2010.
5. Package Insert for CheckCell Antiglobulin Control IgG-Coated Pooled Red Blood Cells, ImmucorGamma, Inc., Norcross, GA, Insert Code 307-16, Revision Date 06/2011.
6. Package Insert for Complement Control Cells for Confirmation of Anti-C3 Reactivity, ImmucorGamma, Inc., Norcross, GA, Insert Code 374-5, Revision Date 09/2010.

18. REVISION HISTORY

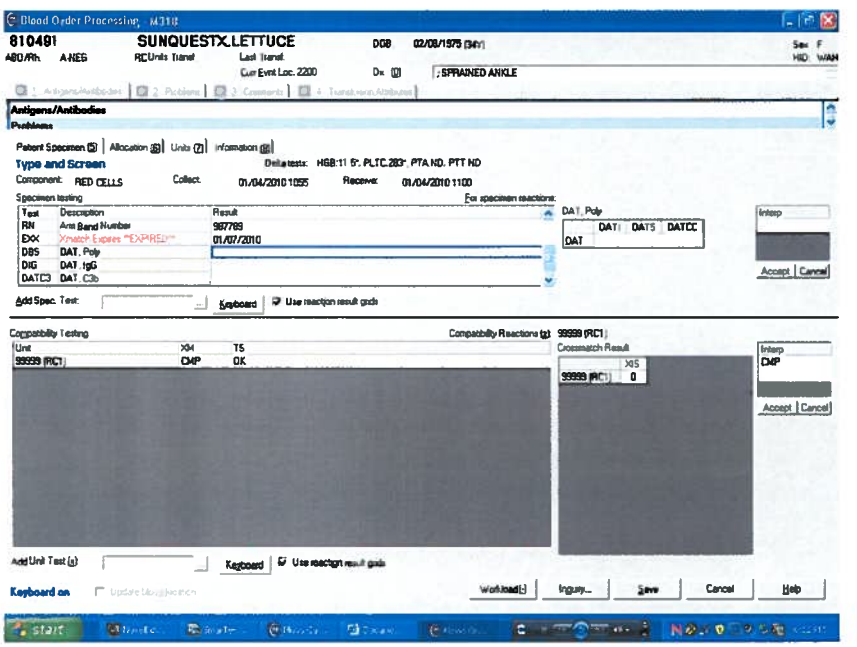
Version	Date	Section	Reason	Reviser	Approval
			Supersedes SOP SWB.003.000		
000	10.27.2011	8.1, 8.2	Updated time of room temperature incubation to state "up to 5 minutes" per the manufacturer's instructions.	SCodina	NCacciabeve
000	10.27.2011	11.2	Title change to local terminology	L Barrett	NCacciabeve
001	11.20.2012	4.1,6.1	Updated reagent order numbers	SCodina	NCacciabeve
001	11.20.2012	6.3	Omitted requirement to test weak positive reactions with check cells	SCodina	NCacciabeve

19. ADDENDA

Addendum	Title
Appendix A	LIS Entry

Appendix A

LIS Entry

Step	Action
1	Access SunQuest function "Blood Order Processing."
2	In the "Lookup by" prompt, click on the dropdown menu and select "Patient ID."
3	In the "Value" prompt, type the patient's medical record number and click on the "Search" button.
4	If more than one patient appears, select the correct patient by clicking on the name.
5	Click on the "Search All" button.
6	Click on the sample with the correct accession number.
7	If the monospecific tests were performed, they need to be ordered. In the "Add Spec Test" field, type A. "DIG" and tab to add the IgG DAT. B. "DATC3" and tab to add the C3 DAT.
8	Click on the test for which results are to be entered.
9	Press the "Home" key to move your cursor to the reaction entry grid.
10	Enter the result of each tube in the appropriate grid box. See the Keypad Map below for specific key entry. A. DATI=Immediate Spin Reading B. DAT5=5 Minute Reading C. DATCC=Check Cells 

	Keypad Map for Result Reactions 7 **H** | 8 **RL** | 9 **NT** 4 **4+** | 5 **M+** | 6 **MF** 1 **1+** | 2 **2+** | 3 **3+** 0 **0** | . **NE** H = Hemolysis RL = Rouleaux NT = Not tested M+ = Microscopic MF = Mixed field NE = Neonatal backtype
11	Enter the interpretation in the "Interp" field. A. "P" = Positive B. "N" = Negative
12	Click on the "Save" button.