TRAINING UPDATE

Lab Location:	SGAH and WAH	**Date Implemented:**	9.28.2012
Department:	Blood Bank	**Due Date:**	10.26.2012

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:
Antibody Titration-SGAH Only
Description of change(s):
• Determine whether the patient has had a previous titer performed during the same pregnancy by searching Laboratory Inquiry • Report a negative titer as "1" if you detect the antibody using neat plasma

EMPLOYEE SIGNATURES

I have read and understand the procedure described above:

Name	Signature	Date

Technical SOP

Title	**Antibody Titration**		
Prepared by	Maria Hall	Date:	12/31/2008
Owner	Stephanie Codina	Date:	05/07/2010

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

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

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1. TEST INFORMATION

Assay	Method/Instrument	Local Code
Antibody Titration	Tube test	BABT

Synonyms/Abbreviations
Antibody Titer

Department
Blood Bank

2. ANALYTICAL PRINCIPLE

Antibody titration can provide useful information about the relative amount of antibody present in a serum. A titer is determined by testing serial dilutions of the serum against a selected red cell sample. Titrations are often performed in prenatal studies when an antibody known to cause hemolytic disease of the fetus and newborn (HDFN) is present; the titration result may aid in assessing the need for amniocentesis.

3. SPECIMEN REQUIREMENTS

Refer to SOP: 'Sample Specifications for Blood Bank Testing' for labeling requirements

3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	Not applicable
Specimen Collection and/or Timing	Not applicable
Special Collection Procedures	Not applicable
Other	Not applicable

3.2 Specimen Type & Handling

Criteria		
Type -Preferred **-Other Acceptable**	EDTA ACD, CPD, CPDA-1, CP2D, oxalate, or clotted blood	
Collection Container	Vacutainer	
Volume - Optimum **- Minimum**	1 ml 1 ml	
Transport Container and Temperature	Transport vacutainer at room temperature or wet ice 1 to 10°C	
Stability & Storage Requirements	Room Temperature:	within 8 hours
	Refrigerated:	1 to 10°C for 48 hours
	Frozen:	Plasma or serum can be stored frozen indefinitely
Timing Considerations	EDTA samples must be tested within 48 hours of collection	
Unacceptable Specimens & Actions to Take	Heparin, sodium citrate, or vacutainers with gel separators are not acceptable and must be recollected.	
Compromising Physical Characteristics	Specimens must be aseptically collected	
Other Considerations	Not applicable	

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	Supplier & Catalog Number
Commercially prepared screening cells	Immucor, Cat.# 2381 or equivalent
Commercially prepared panel cells	Immucor, Cat.# 3023 (Panocell 10), 2332 (Panocell 16), 5020 (Panocell 20) or equivalent
Anti-IgG	Immucor, Cat.# 409210 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	3% Screening Cells (I, II, III), 3% Panel Cell
Container	10ml each
Storage / Stability	1-10°C / Stable until manufacturer's expiration date.
Preparation	Resuspend red cells before use by gently inverting each vial several times.

Reagent	Anti-IgG
Container	10ml
Storage / Stability	1-10°C / Stable until manufacturer's expiration date.
Preparation	Ready to use as supplied.

5. CALIBRATORS/STANDARDS

Not applicable

6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalog Number
Coombs Control cells (IgG coated)	Immucor, Cat.# 2225 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	IgG coated 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 test.

6.4 Tolerance Limits

Reactivity of 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.

6.5 Review Patient Data

N/A

6.6 Documentation

N/A

6.7 Quality Assurance Program

Participation in CAP proficiency testing

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

N/A

7.2 Equipment

37°C dry heat incubator
Automated cell washer
Serofuge
Calibrated timer
Calibrated MLA pipette

7.3 Supplies

Test tubes, (10 x 75 mm and/or 12 x 75 mm)
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 reagents must be reviewed for any changes before the reagent is used.
A current package insert is available in the Reagent Insert binder.

Step	Action
1	Verify that the specimen meets labeling acceptability requirements outlined in procedure, "Sample Specifications for Blood Bank Testing." Specimens from Washington Adventist Hospital will arrive via courier. The specimen will be aliquoted into a freezer tube and must be labeled with the following. A Sunquest specimen label may be used: A. Patient's full name B. Patient's medical record number C. Specimen accession number D. Date E. Initials or tech ID of the person who aliquoted the specimen
2	Titer should not be performed unless antibody screen and antibody identification have been performed within the previous 3 days. A. Antibody screen is necessary to ensure the antibody is still demonstrating in the test specimen. B. Antibody identification is necessary to confirm the antibody identification and ensure no new antibodies have formed.
3	Only titer antibodies that have been associated with HDFN. A. These antibody specificities include: D, C, c, E, e, K, Fy^a, Fy^b, Jk^a, Jk^b, S, s, and M antibodies demonstrating at AHG phase. Consult a supervisor or pathologist if titer of an antibody other than these is requested. B. Never titer Le^a or Le^b antibodies as they are not known to cause HDFN. C. We do not routinely perform hemagglutinin titers (titers of anti-A and anti-B). If a hemagglutinin titer is ordered, please contact a supervisor or pathologist.
4	Order one antibody titer test in Sunquest for each antibody specificity to be titered. Refer to appendix A.

Step	Action
5	Check the LIS to determine whether the patient has had a previous titer tested from the same pregnancy. A. Access Sunquest function "Laboratory Inquiry." B. Lookup by patient MRN or name. C. In the "Number of day (1-9999) prompt, type "300" and tab. D. At the "Code" prompt, type "BTITER" then click the "Add" button. E. Click the"Get Results" button. F. The previous titers will display if present. Pull the most recent sample from the freezer and titer in duplicate with the current specimen. A. Previous titers only need to be performed if more than one titer has been ordered during a pregnancy to determine if the antibody is increasing in titer. B. A previous titer does not need to be repeated if the patient has delivered and is pregnant again.
6	Complete one "Antibody Titration" worksheet for each antibody specificity to be titered. Complete the following blanks: A. Patient's full name B. Patient's medical record number C. Date D. Antibody specificity E. Other antibodies present F. Date of testing G. Tech performing the test
7	Select the 3% reagent red cells to be used for titer testing. A. For patients with multiple antibodies, ensure that the cell used expresses only the antibody to be tested. For example, if the patient has anti-D and anti-K, select a cell that is D+K= to titer anti-D and a cell that is D=K+ to titer anti-K. B. When possible, select a cell with homozygous expression of the antigen. C. Use an R_2R_2 cell when titering anti-D. R_2R_2 cells have the strongest expression of the D antigen.
8	Label 11 tubes according to serum dilution (eg, 1:1, 1:2, 1:4, …1:1024).
9	Pipette 500 µL of saline into each tube **except** the first, 1:1 tube, using a calibrated (MLA) pipette.
10	Pipette 500 µL of patient serum/plasma into the first two tubes (1:1 and 1:2) using a calibrated (MLA) pipette.
11	Mix the contents of the second (1:2) tube well and transfer 500 µL of the diluted serum to the third (1:4) tube. Continue this process through the last tube. Mix well and use a clean pipette tip for each transfer.

Step	Action
12	Label a second set of 11 tubes with the patient identifiers. Refer to procedure, "Sample Specifications for Blood Bank Testing."
13	Label each tube according to dilution (eg, 1:1, 1:2, 1:4, ...1:1024).
14	Add 100µL of diluted plasma to the appropriate tube using a calibrated MLA pipette. (Place 100µL of plasma from the 1:1 tube into the new 1:1 tube, 100µL from the 1:2 tube into the new 1:2 tube, etc). Use a clean pipette tip for each transfer.
15	Add 50µL of reagent red cells to each tube using a calibrated MLA pipette. Refer to step 7 above.
16	**Do not add enhancement.**
17	Gently mix the contents of each tube.
18	Incubate in a 37±2°C heat block for 30 minutes.
19	Wash the tubes a minimum of 4 times with normal saline and decant to a dry button. Use of an automated cell washer is preferred.
20	Add 2 drops of anti-IgG (monospecific) to each tube and gently mix.
21	Serofuge the tubes for the time listed on the serofuge for AHG testing (generally 15 seconds).
22	Beginning with the highest dilution (1:1024) tube, read each tube macroscopically for agglutination using an agglutination viewer. Immediately record results on the "Antibody Titration" form. The titer is reported as the reciprocal of the highest dilution of plasma/serum at which weak positive agglutination is observed.
23	Add 1 drop of Coomb's Control Cells (check cells) to each negative tube (each tube without agglutination) and gently mix.
24	Serofuge each negative tube for the AHG time listed on the serofuge (generally 15 seconds).
25	Read each tube for agglutination. A. Agglutination must demonstrate at strength of 2+ or greater for results to be valid. B. Any check cell result with a strength <2+ is invalid and the test needs to be repeated.

Step	Action
26	If a previous titer was tested, the current titer result should match the original titer within 2 dilutions, or another tech should repeat testing on both previous and current specimens prior to reporting results.
27	Enter results in Sunquest. A. See Appendix B for instructions. B. A negative titer is reported as 1 (not zero) because the antibody was detected in neat plasma.
28	Freeze an aliquot of the current specimen. A. Obtain a freezer tube from the specimen processing area. B. Label the tube with the following information **or** apply a specimen label to the tube. a. Patient's full name b. Patient's medical record number c. Date of specimen d. Specimen number e. Initials of the person aliquoting the specimen C. Place the tube in the bottom bin of the plasma freezer in the rack or bag labeled, "Titer specimens."

9. CALCULATIONS

N/A

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation

The titer is reported as the reciprocal of the highest dilution that produces 1+ macroscopic agglutination. For example, 32 - *not* 1 in 32 or 1:32. If there is agglutination in the tube containing the most dilute serum, the endpoint has not been reached, and additional dilutions should be prepared and tested.

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

See Appendix B for instructions on resulting.

11. EXPECTED VALUES

11.1 Reference Ranges

None established

11.2 Critical Values

None established

11.3 Priority 3 Limit(s)

None established

12. CLINICAL SIGNIFICANCE

Titrations are often performed in prenatal studies when an antibody known to cause HDFN is present; the titration result may aid in assessing the need for amniocentesis. See procedure note 3 below.

13. PROCEDURE NOTES

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

1. Antibodies are unstable in the diluted state. Once the solutions have been made the titration should be tested as soon as possible.

2. The prozone phenomenon may cause reactions to be weaker in the first tubes than in higher dilutions. It is preferable to begin reading with the highest dilution, and proceed to the most concentrated sample.

3. When sequential prenatal serum samples are to be tested for changing antibody titer, 2 mls of the current sample should be frozen for comparison with the subsequent sample. In comparative studies, a two-tube or four-fold difference is considered significant.

4. Enhancement reagents are generally not used when performing titers. Use of albumin, PeG, and LISS will falsely increase results.

5. Failure to change pipette tips after each dilution may cause falsely elevated results.

6. Failure to properly thaw or mix the plasma specimen may yield incorrect results.

14. LIMITATIONS OF METHOD

14.1 Analytical Measurement Range (AMR)

N/A

14.2 Precision

N/A

14.3 Interfering Substances

N/A

14.4 Clinical Sensitivity/Specificity/Predictive Values

N/A

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 learn the 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
Form: Antibody Titration

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. Standards for Blood Banks and Transfusion Services, 2009. AABB, 26th ed. AABB Publishing, Bethesda, Maryland

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
			Supersedes SOP SWB.013.000		
000	5/7/2010		Updated owner	S. Codina	N. Cacciabeve
		11.2	Changed Priority 1& 2 Limits to Critical Values	L. Barrett	N. Cacciabeve
		8	Detailed testing steps added	S. Codina	N. Cacciabeve
		19	Addenda added	S. Codina	N. Cacciabeve
001	11/3/2010	8	Changed amount of plasma added from 2 drops to 100µL and red cells to 50µL; incubation time from 60 min to 30 min; read to W+ instead of 1+	S. Codina	N. Cacciabeve
002	9.25.2012	4.1	Updated to match manufacturer's current item numbers and current panels purchased.	S. Codina	N. Cacciabeve
002	9.25.2012	6.3	Deleted requirement to use check cells with weakly negative reactions.	S. Codina	N. Cacciabeve
002	9.25.2012	8	Updated instructions for searching in LIS for previous titers performed. Added instructions to result a negative titer as 1.	S. Codina	N. Cacciabeve

19. ADDENDA

A. Ordering Antibody Titer Testing in Sunquest
B. Antibody Titer Result Entry

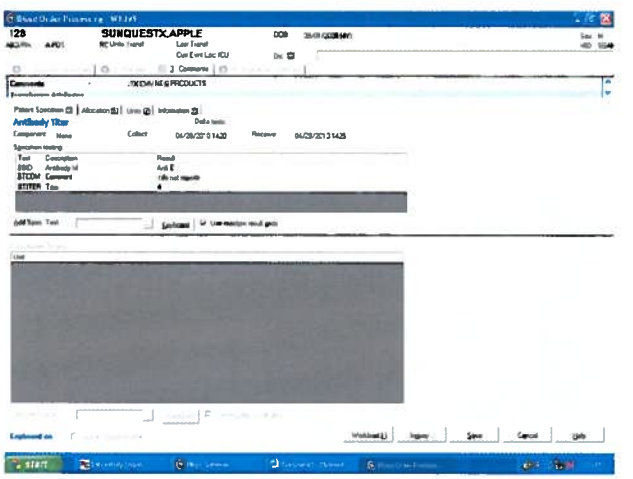
Appendix A

Ordering Antibody Titer Testing in Sunquest

Step	Action
1	Access Sunquest function, "Order Entry."
2	At the "Lookup By" prompt, click on the dropdown menu and select "Patient ID."
3	At the "Value" prompt, type the patient's medical record number and click on the "Search" button.
4	Click on the patient for whom you are ordering testing to highlight then press the "Select" button.
5	At the "Collect Date" prompt, type in the date on which the specimen was collected and press tab. The letter "T" will default with the current date.
6	At the "Collect Time" prompt, type in the time at which the specimen was collected and press tab.
7	At the "Received Date" prompt, type in the date on which the specimen was received and press the tab key. You may press only the tab key to default the current date.
8	At the "Received Time' prompt, type in the time at which the specimen was received and press the tab key. You may press only the tab key to default the current time.
9	At the "Ordering Physician" prompt, type in the number of the patient's physician. This is generally listed at the top of the screen. Alternatively, you can click on the ellipse and search for physician by name. Press tab.
10	At the "Order Code" prompt, type "BABT" and tab.
11	Click on the "Save" button.
12	The titer order will generate a new accession and a label will print.
13	Repeat steps 1-12 for each additional antibody specificity to be titered.

Appendix B

Antibody Titer Result 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	A list of accessions will appear. Look for the accession that corresponds to the antibody titer (BABT).
7	Highlight the correct encounter and press the "Select" button.
8	Enter the results in the appropriate fields. A. In the antibody ID (BBID) field, enter the identity of the antibody and press the tab key. Use the antibody mnemonics listed in procedure, "Antibody Identification." B. If the patient has a previous titer result, the previous result should be entered in the comment (BTCOM) field. For example, "Titer specimen from *date* repeated and titer is *number*." C. In the antibody titer (BTITER) field, enter the titer of the antibody. a. You must type a semicolon (;) before the titer number. b. Titers are reported out as whole numbers (1, 2, 4, …) and not as ratios (1:2, 1:4, 1:8…). c. A negative titer is reported as 1 (not zero) because the antibody was present in the neat plasma. 
9	Click the "Save" button.