TRAINING UPDATE

Lab Location: SGAH **Date Implemented:** 12.18.2012
Department: Blood Bank **Due Date:** 12.31.2012

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:
Pre-warmed Technique
Description of change(s):
▪ Separated procedure into two sections: o Prewarmed AHG test for resolution of antibody problems and AHG XM o Prewarmed IS test for resolution of ABO discrepancies and ISXM ▪ AHG pre-warm uses LISS and 15-30 minute incubation; if still positive, perform pre-warmed without enhancement. ▪ Why? Research shows that clinically-significant antibodies can be pre-warmed away when you use this method without enhancement. Other articles suggest this is caused by using a potentiator (LISS) in one test and no potentiator (no LISS) in the other test. I will place additional reading materials in the BB for anyone who wants to read more about this. ▪ Updated reagent section ▪ Update computer entry

Technical SOP

	Title	**Prewarmed Technique**	
	Prepared by	Leslie Barrett	Date: 2/16/2009
	Owner	Stephanie Codina	Date: 11/21/2012

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	Order Code	Local Code
Prewarmed Antiglobulin Technique	Tube test	PWSC or PWXM	N/A

Synonyms/Abbreviations
N/A

Department
Blood Bank

2. ANALYTICAL PRINCIPLE

Cold-reactive antibodies are often clinically irrelevant. However, they can interfere with serologic testing and mask reactions of more clinically-significant antibodies. The prewarm technique is used to detect clinically-significant antibodies in the presence of cold reactive antibodies. It can be used as a tool for ABO typing, antibody identification, and crossmatching.

3. SPECIMEN REQUIREMENTS

Refer to procedure 'Sample Specifications for Blood Bank Testing' for labeling requirements.

3.1 Patient Preparation

N/A

3.2 Specimen Type & Handling

Criteria		
Type -Preferred **-Other Acceptable**	Preferred: EDTA Other acceptable: ACD, CPD, CPDA-1, CP2D, oxalate, or clotted blood	
Collection Container	Vacutainer	
Volume - Optimum **- Minimum**	1 ml 1 ml	
Transport Container and Temperature	Room Temperature	
Stability & Storage Requirements	Room Temperature:	within 8 hours
	Refrigerated:	1 to 10C 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
ImmuAdd LISS	Immucor, Cat.# 57073 or equivalent
Screening or panel cells	Immucor, Cat.# 2381, 3032 or equivalent
Anti-IgG	Immucor, Cat.# 409210 or equivalent
ABO forward typing sera	Immucor, Cat.#6400, 6406, 6412 or equivalent
ABO reverse typing cells	Immucor, Cat.#2345 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	ImmuAdd, Anti-IgG, ABO forward typing sera
Container	10ml
Storage/Stability	1-10C / Stable until manufacturer's expiration date.
Preparation	Ready to use as supplied.

Reagent	Screening Cells (I, II, III), Panocell-10, ABO reverse grouping cells
Container	10ml each
Storage/ Stability	1-10C / Stable until manufacturer's expiration date.
Preparation	Resuspend red cells before use by gently inverting each vial several times.

5. CALIBRATORS/STANDARDS

N/A

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	1-10C / Stable until manufacturer's expiration date.

6.3 Frequency

With each negative test performed at antiglobulin phase.

6.4 Tolerance Limits

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.

6.5 Review Patient Data

N/A

6.6 Documentation

N/A

6.7 Quality Assurance Program

N/A

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

N/A

7.2 Equipment

Serological centrifuge
37 C dry heat incubator
Timer
37 C waterbath

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.

Prewarm Antiglobulin Technique

Step	Action
1	This technique should never be used as the sole method for antibody identification or crossmatching. Prewarming has been known to warm away clinically-significant antibodies and must be used with caution. Generally speaking, prewarm is not used in our laboratory until after we have ruled out all clinically-significant antibodies. This may require referral of the initial antibody identification to a reference laboratory for testing.
2	Pre-warm all samples and reagents that will be used for testing. A. Pre-warm saline. a. Place a bottle of saline in a plastic bag or overwrap to prevent contamination with water. b. Place the saline in the 37C waterbath for 10-20 minutes. B. Pre-warm the patient plasma. a. Label a test tube with the patient identifiers (patient initials or first 3 letters of the last name). b. Place an aliquot of patient plasma in the tube and incubate at 37°C for 10 minutes C. Pre-warm the test red cells. a. Label a test tube for each red cell to be tested. b. Add one drop of each 2-4% red cell suspension in the appropriate tube. c. Incubate the tubes at 37°C for 10 minutes. D. Pre-warm the LISS. a. Label a test tube with the word "LISS." b. Add an aliquot of LISS to the tube. c. Incubate the tube at 37°C for 10 minutes.

Step	Action
3	Label tests tubes for the testing that will be performed. A. All tubes must contain the patient identifiers (patient initials or first 3 letters of the last name). B. All tubes must contain the reagent identifier. a. For screening or panel cells, the tube is labeled with the cell identification. b. For crossmatches, the tube is labeled with the last 3 digits of the unit number and an X.
4	Place the empty tubes in a 37°C heat block to avoid cooling of the test components.
5	Add 2 drops of warmed patient plasma to each of the tubes.
6	Add 1 drop of the appropriate warmed test cell to each of the labeled tubes.
7	Add 2 drops of warmed LISS to each of the tubes.
8	Mix each tube and continue to incubate at 37°C for 15 minutes (incubation may be extended to a maximum of 30 minutes).
9	Without removing the tubes from the heating block, fill each one with prewarmed saline. Remove tubes from heating block, centrifuge for the posted time, and wash an additional 3 times and completely decant the final wash. (Return the bottle of saline to the waterbath between washes). Refer to procedure, "Manual Wash Technique" for guidance.
10	Add 2 drops of anti-IgG, centrifuge for posted time and read macroscopically using an agglutination viewer. Record reactions immediately and interpret the pre-warmed screen or crossmatch in the computer or on a downtime/antigram form. A pre-warmed panel is recorded on a panel sheet.
11	Add one drop of Coombs Control cells to each negative tube. Mix thoroughly, centrifuge and examine for expected agglutination. Record results. Reactivity 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.
12	If the cold antibody continues to interfere with testing, it may be necessary to repeat the prewarm testing without enhancement.

Prewarm Immediate Spin Technique

Step	Action
1	The immediate spin technique is used to aid in the resolution of ABO discrepancies. Screening cells and an autologous control should be tested simultaneously with ABO/Rh reagents.
2	Prepare a cell suspension using patient cells. A. Pre-warm saline. a. Place a bottle of saline in a plastic bag or overwrap to prevent contamination with water. b. Place the saline in the 37C waterbath for 10-20 minutes. B. Pre-warm the patient red cells. a. Label a test tube with the patient identifiers (patient initials or first 3 letters of the last name). b. Place an aliquot of patient red cells in the tube and incubate at 37°C for 10 minutes. C. Wash the patient red cells using warm saline. a. Without removing the tube containing patient red cells from the heating block, fill with prewarmed saline. b. Remove the tube from the heating block, centrifuge and wash 3 times, completely decant the final wash. c. Return the bottle of saline to the waterbath between washes. d. Refer to procedure, "Manual Wash Technique" for guidance. D. Prepare a 2-4% cell suspension using patients washed cells and warm saline. E. Return the saline bottle to the waterbath.
3	Pre-warm all samples and reagents that will be used for testing. A. Label tubes for each reagent to be tested. a. All tubes should be labeled with the patient's initials or first 3 letters of the last name. b. Each tube should be labeled with the reagent that will be used. i. A for anti-A ii. B for anti-B iii. D for anti-D iv. AC for A_1 cells v. BC for B cells vi. I for screening cell I vii. II for screening cell II viii. III for screening cell III ix. AC for autologous control B. Add 1 drop of the appropriate reagent to each labeled tube. C. Place the tubes in a 37°C dry heat block for 5-10 minutes.

Step	Action
4	Add patient sample to each tube. A. Without removing the labeled tubes from the heat block, a. Add 2 drops of pre-warmed patient plasma to tubes AC, BC, I, II, III, and AC. b. Add 1 drop of pre-warmed patient cell suspension to tubes A, B, D, and AC.
5	Immediately centrifuge the tubes and read macroscopically using an agglutination viewer. Record results immediately on the ABO Discrepancy worksheet.

9. CALCULATIONS

N/A

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

Positive = Agglutination at any strength
Negative = No agglutination

10.2 Rounding

N/A

10.3 Units of Measure

N/A

10.4 Clinically Reportable Range (CRR)

N/A

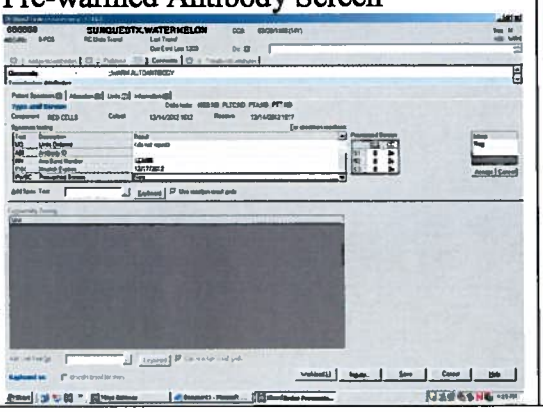
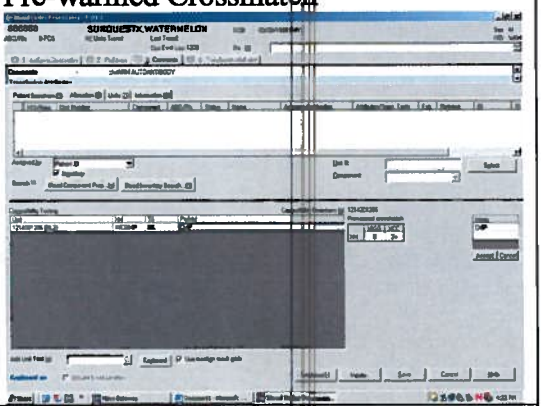
10.5 Repeat Criteria

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

10.6 Result Reporting

Ordering Pre-warmed Testing in Sunquest

Step	Action
1	Access the patient in Sunquest function, "Blood Order Processing" and open the T&S test.

Step	Action
2	To result a pre-warmed antibody screen, A. Type ";PWSC" or "!" in the "Add Spec Test" field to add the test to the workup. B. Highlight the "PWSC" test. C. Press the "Home" key. D. Enter the IgG reading for each of the 3 tubes (S1, S2, S3) in the appropriate fields. E. Enter the check cell readings for each of the 3 tubes in the appropriate fields. F. Interpret the test. a. P is positive b. N is negative
3	To result the pre-warmed crossmatch, A. Type "PWXM" or "@"in the "Add Unit Test" field to add a pre-warmed crossmatch to a unit. B. Enter the IgG and check cell reading results in the appropriate fields. C. Interpret the crossmatch. a. "[" is compatible b. "{" is incompatible
4	Pre-warmed Antibody Screen  Pre-warmed Crossmatch 
	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. EXPECTED VALUES

11.1 Reference Ranges

N/A

11.2 Critical Values

N/A

11.3 Priority 3 Limit(s)

N/A

12. CLINICAL SIGNIFICANCE

Prewarming is useful in determining if cold antibodies are masking the presence of other, clinically-significant antibodies and resolving ABO discrepancies.

13. PROCEDURE NOTES

- **FDA Status:** LDT without message
- **Validated Test Modifications:** None

It is important that the red cells and serum are at 37°C before testing and do not drop below this temperature during incubation or washing. Samples transferred from a 37°C heating block and centrifuged immediately at room temperature may drop 7-8 degrees. Therefore, centrifugation of tests is not recommended after incubation.

14. LIMITATIONS OF METHOD

1. Prewarmed technique should only be used for testing sera after determining that a cold-reactive antibody is present and it reacts at the AHG phase or when a suspected cold antibody is interfering with ABO/Rh typing or immediate spin crossmatching. This technique should never be used as the sole method for ABO/Rh, antibody identification, or crossmatch testing.
2. This technique is not appropriate if no reactivity is observed at the antiglobulin phase during routine testing.
3. In patients who have been transfused or pregnant in the last three months be certain to rule out the presence of IgM antibody before using prewarmed technique.

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 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: Preparing a 2-4% Cell Suspension for Testing
SOP: Blood Bank Reaction Grading
SOP: ABO Discrepancies

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, 27th ed. AABB Publishing, Bethesda, Maryland.

18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
			Supersedes SOP SWB.011.000		
000	11/21/12		Update owner	LBarrett	NCacciabeve
000	11/21/12	11.2	Title change	SCodina	NCacciabeve
	11/21/12	4.2	Updated reagents used for testing	SCodina	NCacciabeve
	11/21/12	8	Added instructions for immediate spin prewarm; added requirement to use enhancement and decreased incubation time to reflect manufacturer's instructions.	SCodina	NCacciabeve
	11/21/12	10.6	Updated LIS entry instructions	SCodina	NCacciabeve
	11/21/12	Addenda	Deleted addenda for determining if antibody reacts at 37C. Capture is our primary testing method; Capture does not detect IgM antibodies.	SCodina	NCacciabeve

19. ADDENDA

None