TRAINING UPDATE

Lab Location:	SGAH and WAH	**Date Implemented:**	1.21.2013
Department:	Blood Bank	**Due Date:**	1.31.2013

DESCRIPTION OF PROCEDURE REVISION

Name of procedure:
Weak D Typing (Manual Tube)
Description of change(s):

• Weak D instructions were removed from the ABO/Rh procedure and moved to a new, Weak D procedure.
• We will now report and interpret the immediate spin D result separately from the weak D result.
  o Report as Rh-positive if IS anti-D result 2+, 3+, or 4+
  o Report as Rh-negative if IS anti-D result is 0
  o Report as Rh-inconclusive if IS anti-D result is W+ or 1+
  o Do NOT wait for weak D results prior to reporting Rh results
  o DO NOT change the ABO/Rh type from negative to positive if the weak D is positive
 	
The ACOG (American Congress for Obstetrics and Gynecology) is recommending that labs interpret and report immediate spin and weak D results separately. There is no standard process between labs for performing and reporting weak D testing. As a result, different labs report Rh results differently for the same patients. The ACOG advises that labs let the physicians interpret these results to ensure the patient gets RhIG whenever indicated. Our OB/Gyn physicians voted to use the ACOG recommendations.

Technical SOP

Title	**Weak D Typing (Manual Tube)**	
Prepared by	Stephanie Codina	Date: 01.13.2013
Owner	Stephanie Codina	Date: 01.13.2013

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

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

TABLE OF CONTENTS

1. TEST INFORMATION

Assay	Method/Instrument	Order Code	Local Code
Weak D Typing	Tube test	N/A	N/A

Synonyms/Abbreviations
Du typing

Department
Blood Bank

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2. ANALYTICAL PRINCIPLE

The descriptive terms Rh-positive and Rh-negative refer to the presence or absence of the red cell antigen D. Most red cell phenotypes have a conventional RhD protein which demonstrates agglutination when mixed with anti-D reagent. However, some people have an RHD allele that codes for a weakened expression of the D antigen. The weakened D antigen requires additional testing to determine whether or not the D antigen is present. Identification of the weak D antigen is required on infants born to Rh-negative mothers to determine RhIG candidacy of the mother. Weak D testing is also used to investigate D typing discrepancies and confirm weak immediate spin D results.

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	Patient identification must be confirmed. Refer to procedure "Sample Specifications for Blood Bank Testing" for details.

3.2 Specimen Type & Handling

Criteria		
Type -Preferred **-Other Acceptable**	Red cells (EDTA) Heparin tube or clotted sample in tube w/out serum separator gel	
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, Clotted samples <21 days
	Frozen:	Unacceptable
Timing Considerations	Test as soon as possible following collection	

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Criteria	
Unacceptable Specimens & Actions to Take	Frozen, Incomplete or incorrect labeling – refer to procedure "Sample Specifications for Blood Bank Testing" for details.
Compromising Physical Characteristic	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.

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.

4.1 Reagent Summary

Reagents / Kits	Supplier & Catalog Number
Anti-D, Series 4	Immucor Cat. #6412 or equivalent
Albumin, 22% Bovine	Immucor Cat. #2327 or equivalent
Anti-IgG	Immucor Cat. #409210 or equivalent
Coombs Control Cells	Immucor Cat. #2225 or equivalent

4.2 Reagent Preparation and Storage

Reagent	Anti-D
Preparation	Ready to use as supplied.
Storage	1-10°C
Stability	Stable until manufacturer's expiration date.
Special Handling	None

Reagent	Albumin, 22% Bovine
Preparation	Ready to use as supplied.
Storage	1-10°C
Stability	Stable until manufacturer's expiration date.
Special Handling	None

Reagent	Anti-IgG
Preparation	Ready to use as supplied.
Storage	1-10°C
Stability	Stable until manufacturer's expiration date.
Special Handling	None

Reagent	Coombs Control Cells
Preparation	Resuspend red cells before use by gently inverting each vial several times.
Storage	1-10°C
Stability	Stable until manufacturer's expiration date.
Special Handling	None

5. CALIBRATORS/STANDARDS

N/A

6. QUALITY CONTROL

6.1 Controls Used

Controls	Supplier and Catalog Number
Ortho Confidence Control Kit	Ortho Clinical Diagnostics, Cat. #32418

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.

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. Refer to the control insert sheet for preparation, storage and handling instructions.

6.3 Frequency

Daily

6.4 Tolerance Limits

Refer to procedure "Daily Reagent Quality Control."

6.5 Review Patient Data

N/A

6.6 Documentation

Refer to procedure "Daily Reagent Quality Control."

6.7 Quality Assurance Program

- Each new shipment and lot number of reagent is tested with control materials before being placed into use. Reagents that do not perform as expected are not placed into use.

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

N/A

7.2 Equipment

Serological centrifuge
Agglutination Viewer
37°C dry heat incubator
Timer

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.

Weak D testing only needs to be performed in the following situations:

A. When testing cord bloods or neonatal types and screens to determine RhIG candidacy of the mother.

B. When the results of the immediate spin Anti-D are positive but yield results that are <2+ in strength.

C. When there is a discrepancy between the patient's current and historical Rh-types (Example: historical Rh is positive and current Rh is negative).

Step	Action
1	Confirm specimen acceptability and specimen labeling per procedure, "Sample Specifications for Blood Bank Testing."
2	Label three tubes with the patient or unit identifiers. Labeling standards are detailed in the policy "Sample Specifications for Blood Bank Testing." Label one tube with each of the following: A. D B. DC
3	Prepare a 2-4% red cell suspension using test cells in tube that contains only patient or unit identifiers. Refer to procedure, "Preparing a 2-4% Cell Suspension for Testing."
4	Add one drop of the blood grouping reagent to the appropriately labeled tube. A. Add 1 drop of Anti-D to the tube labeled "D." B. Add 1 drop of albumin to the tube labeled "DC."
5	Add one drop of the patient cell suspension to each of the tubes labeled "D" and "DC."
6	Mix each tube thoroughly and perform a visual check to ensure reagent volume is correct in each tube.
7	Incubate both tubes at 36-38°C for 15-60 minutes.
8	Wash the tube 3-4 times with isotonic saline. Use of a cell washer is preferred.
9	Add 2 drops of Anti-IgG to each tube and mix thoroughly.
10	Serofuge for the AHG time listed on the serofuge (generally 15 seconds).

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Step	Action
11	Access the patient information data entry screen using Sunquest function "Blood Order Processing" or utilize a computer downtime form.
12	Remove the tubes from the serofuge and verify the labeling of the tubes matches the patient information in the computer or on the downtime form.
13	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. Refer to procedure, "Blood Bank Reaction Grading."
14	Add one drop of Coombs Control cells to each negative tube and mix thoroughly.
15	Serofuge for the AHG time listed on the serofuge (generally 15 seconds).
16	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.
17	Reactivity of Coombs Control 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.

9. CALCULATIONS

N/A

10. REPORTING RESULTS AND REPEAT CRITERIA

10.1 Interpretation of Data

10.1.1 Agglutination represents a positive result in a particular tube while no agglutination represents a negative reaction in a particular tube.

10.1.2 Pattern

Anti-D Read at AHG phase	Albumin Control Read at AHG phase	Coombs Control Cells	Interpretation
No Agglutination	No Agglutination	≥2+ Agglutination	Rh-Negative
<1+ Agglutination	No Agglutination	≥2+ Agglutination	Rh-Indeterminate Consult Supervisor
≥1+ Agglutination	No Agglutination	≥2+ Agglutination	Rh-Positive
Any Result	Any Amount of Agglutination (Positive)	Any Result	Rh-Indeterminate
<1+ Agglutination	No Agglutination	<2+ Agglutination	Wash phase not adequate; repeat testing

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

Refer to section 8, step 17 and Appendix A.

11. EXPECTED VALUES

N/A

12. CLINICAL SIGNIFICANCE

A. Rh-negative mothers who deliver Rh-positive or weak D-positive infants are RhIG candidates.
B. Fetal cell screen testing cannot be performed if the mother and/or infant is weak D positive. Kleihauer-Betke testing must be performed.

13. PROCEDURE NOTES

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

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14. LIMITATIONS OF METHOD

1	Falsely positive or falsely negative test results can occur from bacterial or chemical contamination of test materials, inadequate incubation time or temperature, improper centrifugation, improper storage of materials, or omission of test reagents.
2	Positive reactions obtained with stored specimens may be weaker than those obtained with fresh specimens.
3	Positive results in the weak D test are valid only if the red cells being tested have a negative control/direct antiglobulin test.
4	If a patient has received blood of an Rh-type other than his/her own, it may be difficult to determine the correct Rh type. If a patient with a history of typing as Rh-negative is found to be typing as Rh-positive, obtain a history to see if the patient has received Rh-positive blood. D-negative blood should be transfused if there is any question about the patient's Rh-type.
5	In certain situations, falsely positive results may be obtained in direct tests with anti-D. The D+ red cells of most people will produce strong reactions (3-4+) with monoclonal anti-D. Reactions of less than 2+ in immediate spin tests should be thoroughly evaluated since such reaction may not be due to interaction between reagent anti-D and the D antigen on the test red cells.
6	The presence of strong cold agglutinins or strong rouleaux-forming factors in the serum of a patient could lead to cellular aggregation in tests employing unwashed plasma- or serum-suspended red cells that may be interpreted as agglutination. The same factors often lead to discrepant results in ABO grouping tests using similarly prepared red cells. To determine the validity of positive tests obtained in the presence of potent cold agglutinins or rouleaux-forming proteins, a control of 6–30% albumin should be tested in parallel. Positive results obtained in control tests indicate that those obtained with Anti-D may be invalid. To overcome such problems, test red cells should be thoroughly washed in warm saline and resuspended in saline before testing.

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: Daily Reagent Quality Control
SOP: Sample Specifications for Blood Bank Testing
SOP: Preparing a 2-4% Cell Suspension for Testing
SOP: Blood Bank Reaction Grading

17. REFERENCES

A. Roback, J.D., Grossman, B.J., Harris, T., and Hillyer, C.D. 201. Technical Manual of the AABB, 17th ed. AABB Publishing, Bethesda, Maryland.
B. Standards for Blood Banks and Transfusion Services, 2012. AABB, 28th ed. AABB Publishing, Bethesda, Maryland.
C. Package Insert for Anti-D, Series 4 (Monoclonal Blend), ImmucorGamma, Norcross, GA, Insert 336-8, 8/07.
D. Package Insert for Checkcell (Weak) Antiglobulin Control, ImmucorGamma, Norcross, GA, Insert 307-14, 10/07.
E. Package Insert for Anti-Human Globulin, Anti-IgG (Murine Monoclonal), ImmucorGamma, Norcross, GA. Insert 3001-1, 10/07.

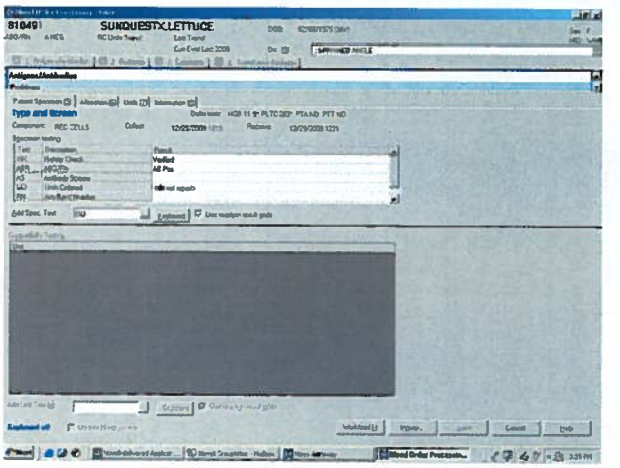
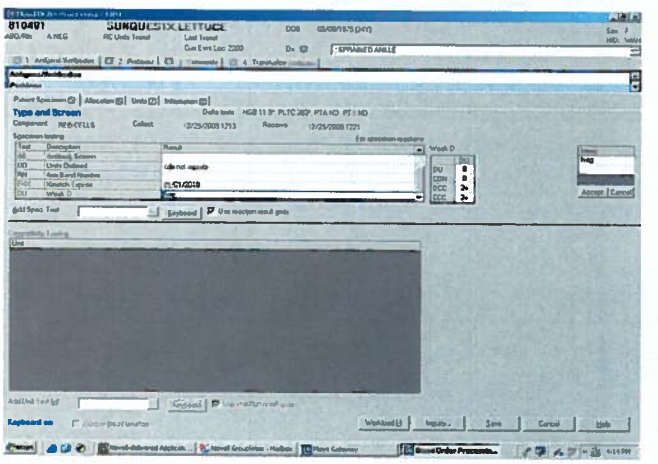
18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval

19. ADDENDA

Appendix A: LIS Entry of Weak D

Appendix A
LIS Entry of Weak D

Step	Action
1	Access the specimen using Sunquest function Blood Order Processing.
2	Order the Weak D test by typing "d" or ";DU" in the "Add Spec Test" field and then pressing the tab button. 
3	Enter the result of each tube in the appropriate grid box. See the Keypad Map below for specific key entry. A. DU = Weak D result at AHG phase B. CON = Albumin control at AHG phase C. DCC=Weak D check cells D. CCC=Albumin control check cells 

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<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>3
Cont.</td><td>Keypad Map
<table>
<tr><td>7
H</td><td>8
RL</td><td>9
NT</td></tr>
<tr><td>4
4+</td><td>5
M+</td><td>6
MF</td></tr>
<tr><td>1
1+</td><td>2
2+</td><td>3
3+</td></tr>
<tr><td colspan="2">0
0</td><td>.</td></tr>
</table>
H = Hemolysis
RL = Rouleaux
NT = Not tested
M+ = Microscopic
MF = Mixed field</td></tr>
<tr><td>4</td><td>Interpret the results of the weak D test.
A. “N” = negative weak D
B. “P” = positive weak D
C. “I” = Indeterminate weak D</td></tr>
</table>

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