#### TRAINING UPDATE

**Lab Location:** 

SGAH and WAH

**Date Implemented:** 

11.1.13

Department:

Blood Bank

**Due Date:** 

11.15.13

### **DESCRIPTION OF PROCEDURE REVISION**

# Name of procedure:

Cord Blood Evaluation/Neonatal DAT

### Description of change(s):

- 1. Added picture of "clumps" in cell suspension with a warning to avoid dripping clumps into tubes used for testing
- 2. Added requirement to leave a blank row between patients in the rack
- 3. Deleted the appendix that listed how to receive a sample; refer the user to the new procedure for receiving samples

3/14/2010

Date:

Technical SOP				
Title	Cord Blood Evaluation / Neonatal DAT			
Prepared by	Stephanie Codina	Date:	3/14/2010	

Stephanie Codina

Owner

Laboratory Approval	Local Effective Da	ite:
Print Name	Signature	Date
Refer to the electronic signature page for approval and approval dates.		
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Review		
Print Name	Signature	Date
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# 1. TEST INFORMATION

Assay	Method/Instrument	Order Code	Local Code
Cord Blood Evaluation Neonatal ABO /Rh and DAT	Tube test	CORDEV NDAT	N/A

Synonyms/Abbreviations	
Type and Direct Coombs	

Department	
Blood Bank	

### 2. ANALYTICAL PRINCIPLE

Cord blood evaluation and neonatal DAT batteries consist of two tests: ABO/Rh and DAT.

- ABO/Rh Used to determine RhIG candidacy of the newborn's mother. A red cell suspension is mixed with certain antisera to demonstrate the presence or absence of agglutination. The subsequent pattern of agglutination is utilized to determine the ABO and Rh groups.
- DAT Used for the investigation of hemolytic disease of the newborn. Red cells are washed and mixed with IgG anti-human globulin to determine whether they are coated in-vivo with immunoglobulin.

# 3. SPECIMEN REQUIREMENTS

# 3.1 Patient Preparation

Component	Special Notations
Fasting/Special Diets	None
Specimen Collection and/or Timing	None
Special Collection Procedures	None
Other	N/A

### 3.2 Specimen Type & Handling

Criteria		
Type -Preferred	Red cells and plasma (EDTA)	
-Other Acceptable	Clotted sample in tube without serum separator gel	
Collection Container	Lavender bullet	
Volume - Optimum	Cord blood – 5 ml, Heel stick – 2ml	
- Minimum	1ml	
Transport Container and	Same as above at room temperature	
Storage		
Stability & Storage	Room Temperature: 24 hours	
Requirements	Refrigerated: EDTA samples <10 days,	
	Frozen: Unacceptable	
Timing Considerations	Test as soon as possible following collection	
Unacceptable Specimens	Frozen, Incomplete or incorrect labeling – see below.	
& Actions to Take	Reject specimen, notify nursing unit to re-collect.	
Labeling	Specimen must contain the infant's name, infant's medical	
	record number, date and time of collection, and the	
	collector's identification. The infant's name will appear in	
	the following format:	
	A. Mother's last name, sex of child, mother's first	
	name. Example: Doe,BoyJane	
	B. Multiples will be differentiated using letters.	
	Example: Doe, ABoy Jane or Doe, BGirl Jane	

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#### 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-A	Immucor Anti-A, Cat.#6400, or equivalent	
Anti-B	Immucor Anti-B, Cat.#6406, or equivalent	
Anti-D (monoclonal blend)	Immucor Anti-D, Cat.#6412, or equivalent	
Anti-IgG	Immucor, Cat.# 409250 or equivalent	
Coombs Control cells (IgG coated)	Immucor, Cat.# 2225 or equivalent	
Albumin, 22% Bovine	Immucor Cat. #2327 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-A, Anti-B, Anti-D, Anti-IgG, and 22% Albumin	
Container	10ml	
Storage	1-10°C	
Stability	Stable until manufacturer's expiration date.	
Other Do not use if turbid - indicates deterioration or contamin Do not use leaking vials.		
Preparation	Ready to use as supplied.	

Reagent	Coombs Control Cells
Container	10ml
Storage	1-10°C
Stability	Stable until manufacturer's expiration date.
Other	Do not use contaminated or leaking vials.
	Date and initial all reagents upon opening.
	Each container must be labeled with substance name, lot
	number, date of preparation, expiration date, tech initials, special storage instructions.

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Preparation	Resuspend red cells before use by gently inverting each vial several times.
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## 5. CALIBRATORS/STANDARDS

N/A

# 6. QUALITY CONTROL

#### 6.1 Controls Used

Controls	Supplier and Catalog Number
Ortho Confidence Kit	Ortho, Cat.# 6902096

# 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.

# 6.3 Frequency

Daily

# 6.4 Tolerance Limits

Refer to procedure "Reagent Quality Control."

#### 6.5 Review Patient Data

N/A

# 6.6 Quality Assurance Program

Participation in CAP proficiency testing.

### 7. EQUIPMENT and SUPPLIES

### 7.1 Assay Platform

Manual Tube Testing

# 7.2 Equipment

Serological centrifuge Automated cell washer 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.

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.

8.1 ABO/Rh and DAT Testing

Step	Action			
1	Confirm specimen acceptability and specimen labeling per procedure, "Sample Specifications for Blood Bank Testing."			
2	Order testing per procedure, "Order Entry, Entering Orders in the GUI System" or receive the order per procedure, "Order Entry, Receiving Orders in the GUI System."			
3	Perform a history check per procedure, "Patient History Check."  A. The history check is performed on the infant's mother.  B. Blood banks staff will automatically order and test a cord evaluation if the infant's mother has a clinically-significant antibody.			
4	Label five tubes with the patient identifiers. At a minimum, tubes must contain the first 3 letters of the patient's last name or the patient's initials. Labeling standards are detailed in the policy "Sample Specifications for Blood Bank Testing."			
5	Label each tube with one of the following:  A. "A"  B. "B"  C. "D"  D. "DAT" or "IgG  The fifth tube will only contain the patient identifiers for cell suspension.			

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Step	Action		
6	Arrange tubes in a row in a test tube rack. Allow an empty row between the tubes for each patient specimen.		
7	Prepare a 2-4% suspension of patient or donor red cells in isotonic saline in the remaining tube per procedure, "Preparing a 2-4% Cell Suspension for Testing." Ensure the cell suspension does not contain clots or fibrin. This can be done by swirling 2 wooden sticks in the cell suspension and removing the clots and clumps.		
8	Place 1 drop of the red cell suspension in each tube labeled A, B, D, and DAT or IgG.		
	Look at the barrel of the pipette when dripping the cell suspension. Ensure that NO clots or clumps are transferred to the test tubes.		
9	Wash the tubes a minimum of 3 times with isotonic saline. The final wash should be decanted to yield a dry cell button. Use of an automated cell washer is preferred.		
	Note: The wash step may be omitted for ABO grouping of heel stick specimens. Cells used for DAT testing must be washed a minimum of three times prior to testing.		
10	Add one drop of reagents to the following tubes for ABO typing.		
	A. Add 1 drop of anti-A to the tube labeled "A."		
	B. Add 1 drop of anti-B to the tube labeled "B."		
	C. Add 1 drop of anti-D to the tube labeled "D."		
NOTI steps	E: The remaining steps should be performed for ONE patient at a time. Complete 8-13 for one patient and then return to step 8 for the next patient.		
11	Add 2 drops of anti-IgG to the tube labeled "DAT" or "IgG."		
12	Mix each tube thoroughly and perform a visual check to ensure reagent volume is correct in each tube.		

Step	Action			
13	Serofuge for the saline phase calibration time.  A. Serofugation must take place immediately after adding anti-IgG to the tube.  B. If the test system is not serofuged within 1 minute of adding the anti-IgG reagent, the results are considered invalid and test must be repeated.			
14	Access the patient information data entry screen in Sunquest in function "Blood Order Processing" or utilize a computer downtime form.			
15	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.			
16	Gently resuspend the tubes and read reactions macroscopically using an agglutination viewer. Record results as they are read.			
17	If the anti-IgG tube is  A. Positive, interpret the DAT as positive.  B. Negative,  i. Add 1 drop of Coombs Control Cells and gently mix.  ii. Serofuge for the saline phase calibration time.  iii. 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.  iv. If the tube is  i. Positive for agglutination at a strength ≥2+ after addition of Coombs Control cells, interpret test as negative. No further testing is indicated.  ii. Negative or positive for agglutination at strength <2+ after the addition of Coombs Control cells, the test is invalid. Repeat testing beginning at step 1.			

Step	Action			
18	If the infant is AB-Positive, an albumin control must be run.			
	A. Label one test tube with the patient's identifiers and "ALB."			
	B. Add 1 drop of 22% albumin to the tube.			
	C. Add 1 drop of the infant's cell suspension to the tube.			
	D. Mix the tube thoroughly and perform a visual check to ensure reagent volume is correct in each tube.			
	E. Serofuge for the saline phase calibration time.			
	F. 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.			
	G. Gently resuspend the tubes and read reactions macroscopically using an agglutination viewer. Record results as they are read.			
	i. If results are negative, interpret the ABO group as "AB-positive" and continue.			
	ii. If results are positive, wash one drop of the infant's cell suspension a minimum of 3 times in isotonic saline and repeat steps 15A-15G omitting step C.			
	i. If results are negative, interpret the ABO group as "AB-positive and continue.			
	<ul><li>ii. If results are positive, the infant's blood type is invalid and cannot be interpreted. Go to procedure, ABO Discrepancy Resolution.</li></ul>			

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Step	Action	
19	Perform reflex testing as needed.  A. Weak D testing must be performed on any infant that meets any of the following conditions. Refer to procedure, "Weak D Typing (Manual Tube)."  1) Who was born to an Rh-negative mother and is Rh-negative after immediate spin testing to determine RhIg candidacy of the mother.  2) Whose immediate spin anti-D yields results <2+ in strength.  3) Note: Occasionally, you will obtain an inconclusive result for weak D testing when the infant is Rh-negative with a positive DAT. This is most often seen when the mother is O-negative and the infant is A- or B-negative.  a. Notify the patient care area. They may choose to collect a heel stick specimen for testing. This may resolve the inconclusive result.  b. If the Rh is reported as inconclusive (including situations in which the patient care area chooses not to collect a heel stick specimen) the moth should be considered a RhIG candidate and a fetal cells screen test should be ordered.  B. Perform an eluate and eluate antibody identification (if applicable) per procedure, "Acid Elution" on any infant:  1) Whose mother currently has a clinically-significant antibody.  2) Whose positive DAT result cannot be explained by ABO incompatibility or passive transfer of RhIG from mother to baby.  3) Acid elution is NOT normally performed on infants born to mother whose plasma contains only passive anti-D due to RhIG administration. However,	
	elution may be performed by physician request.	
20	Call positive cord blood DAT results to the appropriate nursing unit. Document the A. Person notified B. Date/time called C. Test called	
21	Cord blood and heel stick specimens are stored for a minimum of 10 days and are then discarded.	

8.2 ABO/Rh Retype (Confirmation) Testing

Step	Action
1	ABO/Rh retype (ABR) testing should not be performed by the same technologist performing the original ABO typing whenever possible.
2	Label four tubes with the patient or unit identifiers. Labeling standards are detailed in the policy "Sample Specifications for Blood Bank Testing."

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Step	Action			
3	Label each tube with one of the following:			
	A. "A"			
	B. "B"			
	C. "D"			
	The fourth tube will only contain the patient identifiers.			
4	Add one drop of reagent to the appropriately labeled tube.			
	A. Add 1 drop of Anti-A to the tube labeled "A."			
	B. Add 1 drop of Anti-B to the tube labeled "B."			
	C. Add 1 drop of Anti-D to the tube labeled "D."			
5	Prepare a 2-4% suspension of patient or donor red cells in isotonic saline in the			
	remaining tube per procedure, "Preparing a 2-4% Cell Suspension for Testing."			
	The cord blood suspension may be washed prior to testing, but washing is not required			
6	Add one drop of the patient cell suspension to the tubes labeled "A," "B," and "D."			
7	Mix each tube thoroughly and perform a visual check to ensure reagent volume is correct in each tube.			
8	Serofuge for the saline phase calibration time.			
9	Access the patient information data entry screen in Sunquest in function "Blood Order Processing" or utilize a computer downtime form.			
10	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.			
11	Read tubes macroscopically for agglutination using an agglutination viewer. Record results immediately in the LIS or on a downtime form.			
12	Repeat ABO/Rh testing using washed red cells if the ABO/Rh of the retype does match the original ABO/Rh of the specimen. Have a third tech repeat testing if the discrepancy does not resolve.			
13	Weak D testing is not required for ABO retype testing on cord blood and heelstick specimens unless a discrepancy exists between the two samples (i.e. the baby's initial type was reported out as Rh-positive due to a weak D and the retype result is negative at immediate spin).			

# om revised 10.51.0

# 9. CALCULATIONS

N/A

# 10. REPORTING RESULTS AND REPEAT CRITERIA

# 10.1 Interpretation of Data

# ABO/Rh

Anti-A	Anti-B	Anti-D	Albumin Control	Interpretation
0	0	0	N/A	O-negative, perform weak D testing if mom is Rh-negative
0	0	<u>≥</u> 2+	N/A	O-positive
≥2+	0	0	N/A	A-negative, perform weak D testing if mom is Rh-negative
<u>≥</u> 2+	0	>2+	N/A	A-positive
0	≥2+	<u>≥</u> 2+ 0	N/A	B-negative, perform weak D testing if mom is Rh-negative
0	<u>≥</u> 2+	≥2+ 0	N/A	B-positive
≥2+	<u>≥</u> 2+	0	N/A	AB-negative, perform weak D testing if mom is Rh-negative
<u>≥</u> 2+	<u>≥</u> 2+	<u>≥</u> 2+	0	AB-positive
<u>≥</u> 2+	≥2+	≥2+	≥2+	Invalid Results, refer to ABO Discrepancy Resolution procedure
Any result that is positive but <2+ in strength				Invalid Results, refer to ABO Discrepancy Resolution procedure
Any mixed-field result				Indicates possible contamination with mom's blood A. Request a heel stick specimen if cord blood was used. B. Refer to ABO Discrepancy Resolution Procedure if heel stick blood was used.

#### **DAT**

Anti-IgG	Coombs Control Cells	Interpretation
+	N/A	Positive
0	≥2+	Negative
0	<2+ or 0	Invalid, Repeat

### 11. EXPECTED VALUES

N/A

# 12. CLINICAL SIGNIFICANCE

- RhIg administration is indicated when an Rh-negative mother delivers an Rh-positive infant.
- Negative or positive DAT results can indicate hemolysis is occurring in a case of hemolytic disease of the newborn.

# 13. PROCEDURE NOTES

• FDA Status: Approved/cleared

• Validated Test Modifications: None

# 14. LIMITATIONS OF METHOD

1	DAT testing should not be performed on cord blood samples >72 hours old. If requested, a heel stick specimen should be collected.
2	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.
3	Certain subgroups of A and B may produce reactions that are weaker than those routinely obtained with A or B cells.
4	Infants <6 months in age do not normally produce ABO antibodies. Presence of ABO antibodies in a cord sample can indicate contamination with mother's blood. Interpretation of results cannot be made. A heelstick specimen should be requested when cord blood contamination is suspected.
5	Cord blood samples may contain maternal anti-A and/or anti-B and is not used routinely for reverse grouping.
6	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).
7	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.
8	Positive Coombs Control Cells does not provide absolute assurance that false results will not occur.

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#### 15. SAFETY

The employee has 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.

Report all accidents and injuries <u>immediately</u> to your supervisor or the business unit Environmental Health and Safety Manager or Specialist.

#### 16. RELATED DOCUMENTS

SOP: Order Entry, Entering Orders in the GUI System

SOP: Order Entry, Receiving Orders in the GUI System

SOP: Sample Specifications for Blood Bank Testing

SOP: Patient History Check

SOP: Preparing a 2-4% Cell Suspension for Testing

SOP: Weak D Typing (Manual Tube)

SOP: Acid Elution

#### 17. REFERENCES

- A. Roback, J.D., Grossman, B.J., Harris, T., and Hillyer, C.D. 2011. Technical Manual of the <u>AABB</u>, 17<sup>th</sup> ed. AABB Publishing, Bethesda, Maryland.
- B. Standards for Blood Banks and Transfusion Services, 2012. AABB, 28<sup>th</sup> ed. AABB Publishing, Bethesda, Maryland.
- C. Package Insert for Anti-A, Anti-B (Murine Monoclonal), Anti-A,B (Murine Monoclonal blend), Immucor Inc., Norcross, GA, Insert Code 3006-1, Revision Date 10/2007.
- D. Package Insert for Anti-D, Series 4 (Monoclonal Blend), ImmucorGamma, Norcross, GA, Insert 336-8, 8/07.
- E. Package Insert for Anti-IgG (Murine Monoclonal), ImmucorGamma, Inc., Norcross, GA, Insert Code 3001-1, Revision Date 10/2007.
- F. Package Insert for CheckCell Antiglobulin Control IgG-Coated Pooled Red Blood Cells, ImmucorGamma, Inc., Norcross, GA, Insert Code 307-14, Revision Date 10/2007.

# 18. REVISION HISTORY

Version	Date	Section	Reason	Reviser	Approval
			Supersedes SOP SHB/WAB.006.000		
000	4.23.2011	8	Added requirements to check mom's history in the LIS and for fibrin and clots in specimen. Included instructions for reporting inconclusive weak D results due to positive DAT.	SCodina	NCacciabeve
001	8.2.2012	3.2	Updated specimen labeling requirements per new hospital guidelines.	SCodina	NCacciabeve
		19 App A 19 App B	Updated specimen receipt procedure to utilize GUI instead of Smarterm.		
		15 11pp 15	Added ABO retype to the NDAT battery.		
002	10.15.13	8.1	Added requirement to leave a blank row between patients in test tube rack; added picture of "clumps" with warning for pipetting. Minor wording updates for clarity.	SCodina	NCacciabeve
		16, 19	Deleted appendix A and refer user to the new procedure for sample receipt. Updated appendix B.		
		Footer	Version # leading zero's dropped due to new EDCS in use as of 10/7/13.		

# 19. ADDENDA

Appendix A: Entering Cord Evaluation and Neonatal DAT Results in the LIS

# Appendix A Entering Cord Evaluation and Neonatal DAT Results in the LIS

Step				Action
1	Access Sunquest fu	est function "Blood Order Processing."		
2	In the "Lookup by"	by" prompt, click on the dropdown menu and select "Patient ID."		
3		prompt, type the patient's medical record number and click on the		
4	If more than one pa	tient appea	ars, sel	ect the correct patient by clicking on the name.
5	Click on the "Searc			
6	A list of accessions blood evaluation (C	A list of accessions will appear. Look for the accession that corresponds to the cord blood evaluation (CORDEV) or neonatal DAT (NDAT).		
7				press the "Select" button.
	In the ABR field, press the "Home" key to move your cursor to the reaction entry gr Enter the result of each tube in the appropriate grid box. See the keypad map below specific key entry.  A. A = Anti-A result B. B = Anti-B result C. A1C = A <sub>1</sub> cell result; type a period (.) to indicate the test was performed on an infant <6 months of age. D. BC = B cell result; type a period (.) to indicate the test was performed on an infant <6 months of age. E. D = Anti-D result F. CON = albumin control result  Keypad Map for Result Reactions			type a period (.) to indicate the test infant <6 months of age. pe a period (.) to indicate the test infant <6 months of age. rol result
	7	8	9	H = Hemolysis
	H 4	<b>RL</b> 5	NI	<del></del>
	4+	1	6 M1	NT = Not tested M+ = Microscopic
	1	2	3	MF = Mixed field
	1+		3+	
		0	Note:	
9	Enter the blood type Keyboard			tion field.
		Keybo	ard	Sunquest Translation
		A		Group A
		Q Q		Crown D

Keyboard	Sunquest Translation
Α	Group A
В	Group B
C	Group AB
0	Group O
N	Negative
P	Positive
I	Indeterminate

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Step	Action
10	In the DIG field, press the "Home" key to move your cursor to the reaction entry grid.  Enter the result of each tube in the appropriate grid box. See the keypad map below for specific key entry. Use the Keypad Map above.  A. DATI = Immediate spin IgG result  B. DATCC = Coomb's control cell (check cell) result
11	Refer to step 9 for ARC entry instructions. The ARC is an abbreviated entry of the ABR. This field should be tested and entered by a different tech.    Compare   Comp
12	Complete the MMR field with the mother's medical record number.  A. If the sample order was generated using the hospital system, the mother's medical record will automatically fill into the MMRN field.  B. If the sample order was generated in Sunquest, obtain the mother's medical record number from the CHOLD order and type it into the MMR field of the cord evaluation.
13	<ul> <li>Add a comment to explain results if the IgG DAT was positive or if critical results were called.</li> <li>A. In the "Add Spec Test" field, type ";BBCMT" and press the "Tab" key to add a blood bank comment field.</li> <li>B. In the comment field, type two semicolons (;;) then free text the appropriate comment, for example: <ol> <li>"Mom O-positive, baby A-positive"</li> <li>"Anti-C identified in mom"</li> <li>"Mom received RhIg on date"</li> </ol> </li> </ul>

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Step	Action
14	Add a call note if critical results were called to the patient care area.
	A. In the "Add Spec Test" field, type ";BBCALL" and press the "Tab" key to add the entry field.
	B. IN the "BBCALL" field, type two semicolons (;;) then free text the appropriate comment, for example, "Positive cord blood DAT reported to name at time."
15	Click on the "Save" button.

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