Tec	hnic	al S	OP
100	TITLE	m u	\sim

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

Laboratory Approval	Local Effective Da	ite:
Print Name	Signature	Date
Refer to the electronic signature page for approval and approval dates.		
		9

Print Name	Signature	Date
- C		
9 8		
7 10		

TABLE OF CONTENTS

1.	Test Information	
2.	Principle	
3.	Specimen Requirements	
4.	Reagents	
5.	Calibrators/Standards	
6.	Quality Control	
7.	Equipment And Supplies	6
8.	Procedure	6
9.	Calculations	
10.		
11.	Expected Values	
12.	Clinical Significance	
	Procedure Notes	
	Limitations Of Method	
	Safety	
	Related Documents	
	References	
	Revision History	
	Addenda	

1. TEST INFORMATION

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

0.1.	
Criteria	
Type -Preferred	Red cells and plasma (EDTA)
-Other Acceptable	Clotted sample in tube without serum separator gel
Collection Container	Lavender or red top bullet, or red top tube
Volume - Optimum	Cord blood - 10ml, 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,
	Clotted samples <3 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 for Cord Blood	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
10	the following format:
	A. Mother's last name, sex of child, mother's first
-	name. Example: Doe,BoyJane
, a	B. Multiples will be differentiated using letters.
	Example: Doe, ABoy Jane or Doe, BGirl Jane

Criteria	
Labeling for Neonatal T&S	Specimen will be labeled using the blood bank labeling system. Refer to procedure, "Sample Specifications for Blood Bank Testing."

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.		

SOP ID: SGAH.BB40

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.
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		
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.

Form revised 10/51/02

7. EQUIPMENT and SUPPLIES

7.1 Assay Platform

Manual Tube Testing

7.2 Equipment

Serological centrifuge Automated cell washer Timer

7.3 Supplies

10 x 75 mm or 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	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.
3	Label five tubes with the patient or unit identifiers. Labeling standards are detailed in the policy "Sample Specifications for Blood Bank Testing."
4	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.

Step	Action	
5	Prepare a 2-4% suspension of patient or donor red cells in isotonic salir remaining tube per procedure, "Preparing a 2-4% Cell Suspension for T Ensure the cell suspension does not contain clots or fibrin. This can be swirling 2 wooden sticks in the cell suspension and removing the clots a	esting." done by
6	Place 1 drop of the red cell suspension in each tube labeled A, B, D, and	DAT or IgG.
7	Wash the tubes a minimum of 3 times with isotonic saline. The final w decanted to yield a dry cell button. Use of an automated cell washer is	
	Note: The wash step may be omitted for ABO grouping of heel stick spused for DAT testing must be washed a minimum of three times prior to	pecimens. Cells testing.
8	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 ting 8-13 for one patient and then return to step 8 for the next patient.	ne. Complete
9	Add 2 drops of anti-IgG to the tube labeled "DAT" or "IgG."	
10	Mix each tube thoroughly and perform a visual check to ensure reagent correct in each tube.	volume is
11	Serofuge for the saline phase calibration time. A. Serofugation must take place immediately after adding anti-IgG to t B. If the test system is not serofuged within 1 minute of adding the anti the results are considered invalid and test must be repeated.	
12	Access the patient information data entry screen in Mysis in function "Processing" or utilize a computer downtime form.	Blood Order
13	Remove the tubes from the serofuge and verify the labeling of the tubes patient information in the computer or on the downtime form.	matches the
14	Gently resuspend the tubes and read reactions macroscopically using an viewer. Record results as they are read.	agglutination

Page 7 of 19

Step	Action
15	If the anti-IgG tube is A. Positive, interpret the DAT as positive. B. Negative, 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 testing beginning at step 1.
16	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. 1) If results are negative, interpret the ABO group as "AB-positive" and continue. 2) 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. a) If results are negative, interpret the ABO group as "AB-positive" and continue. b) If results are positive, the infant's blood type is invalid and cannot be interpreted. Go to procedure, ABO Discrepancy Resolution.

Step	Action
17	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, "Rh Testing (Tube Method)." 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 ≤1+ 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 mother 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 cannot be explained by an ABO incompatibility between the infant and the mother or by passive transfer of RhIg from mother to baby. C. Acid elution is not normally performed on infants born to mothers whose plasma contains only passive anti-D due to RhIg administration. However, elution can be performed at the request of a physician.
18	Call positive cord blood DAT results to the appropriate nursing unit. Document the A. Person notified B. Date/time called C. Test called
19	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 tec performing the original ABO typing whenever possible.	hnologist
2	Label four tubes with the patient or unit identifiers. Labeling standards the policy "Sample Specifications for Blood Bank Testing."	are detailed in

е	
ď	

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 Mysis 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).

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

SOP ID: SGAH.BB40

CONFIDENTIAL: Authorized for internal use only

SOP Version # 002

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: Sample Specifications for Blood Bank Testing

SOP: Rh Type SOP: Acid Elution Current Package Inserts:

Anti-A, Anti-B (Murine Monoclonal), Anti-A,B (Murine Monoclonal blend), Immucor Inc.

Anti-D, Series 4 (Monoclonal Blend), ImmucorGamma, Inc.

Anti-IgG (Murine Monoclonal), ImmucorGamma, Inc.

CheckCell Antiglobulin Control IgG-Coated Pooled Red Blood Cells, ImmucorGamma, Inc.

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.
- 3. 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.
- 4. Package Insert for Anti-D, Series 4 (Monoclonal Blend), ImmucorGamma, Norcross, GA, Insert 336-8, 8/07.
- 5. Package Insert for Anti-IgG (Murine Monoclonal), ImmucorGamma, Inc., Norcross, GA, Insert Code 3001-1, Revision Date 10/2007.

SOP ID: SGAH.BB40

6. 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	Updated specimen receipt procedure to utilize GUI instead of Smarterm.		
	. W	App D	Added ABO retype to the NDAT battery.		

19. ADDENDA

Appendix A: Receiving Cord Blood Specimens in the LIS

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

Appendix A

Receiving Cord Blood Specimens in the LIS

Step	Action
1	Determine the specimen accession number via the pending log or OL monitor.
2	Access Sunquest GUI function "order entry."
3	At the "Lookup Mode" prompt, A. Select "Acc #" from the dropdown menu. B. Enter the specimen accession number in the box. C. Click on the "search" button.
	D. Select the appropriate accession from the list. E. Click the "select" button.
4	Verify that mom's medical record number is both entered and entered correctly in the modifier column on the right side of the screen. A. If mom's medical record number is missing or incorrect, a. Cancel the CORDEV order per SOP "Canceling Orders." b. Ask the floor to place a new order with the correct mom's MRN. c. Do not discard the specimen. The specimen can be used with the new order if labeled correctly. B. If mom's medical record number is correct, a. Record the MRN on the specimen.
	b. Proceed with step 5.
5	At the "Collect Date" prompt, type the date on which the specimen was collected and press enter. This should be obtained from the specimen label.
6	At the "Collect Time" prompt, type the time at which the specimen was collected and press enter. This should be obtained from the specimen label.
7	At the "Received Date" and "Received Time" prompts, press enter to default the current date and time.
8	At the "Ordering Physician" prompt, press enter to accept the physician that was entered by nursing staff at the time the order was placed.
9	At the "Phleb Code" prompt, type "870" for physician collected and press enter.
10	At the "Phleb Workloadd" prompt, type "MDC" and press enter.
11	Print a barcode label for the specimen. a. Click on the "Reprint Labels" box. b. The "Reprint Labels" pop-up box will appear. c. Select the specimen label from the list or click the "select all" button. d. Click "Print."
	e. The label will print.
12	Click the "save" button to save the specimen receipt data.

Appendix B Entering Cord Evaluation and Neonatal DAT Results in the LIS

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 cord blood evaluation (CORDEV) or neonatal DAT (NDAT).
7	For Cord Blood Evaluation Specimens only: Compare the mother's medical record number that is listed in the "Modifier" column to mother's medical record number on the specimen. A. Nursing staff manually enters this number when ordering the cord blood i the LIS. B. The results may only show the mom's medical record number. C. You can expand the modifier field by clicking on the bar to the right of the word modifier and dragging the box to the right. D. Cancel the order if the medical record numbers do NOT much match exactly or if mom's medical record number is missing. Call the unit and
	request that the order be cancelled and re-entered using the correct mother's medical record number. There is no need to collect a new specimen; the original specimen can still be used.
	mother's medical record number. There is no need to collect a new