

## CORD BLOOD TESTING

Test Code: CORD

### I. PRINCIPLE

Umbilical cord bloods are collected and sent to the lab on all newborns. Some will have an order for an ABO, Rh typing, and Direct Antiglobulin Testing (DAT). Others are stored just in case testing is added on at a later time.

### II. CLINICAL SIGNIFICANCE

Typing is done to assist in determining if a Rh negative mother needs Rh immune globulin or to assist in the diagnosis of Hemolytic Disease of the Fetus and Newborn (HDFN). In the case of HDFN, a Direct Antiglobulin Test or Direct Coombs is also helpful.

### III. SPECIMEN

- A. Umbilical cord blood or anticoagulated (EDTA) specimen.
  - 1. All specimens are kept for 10 days stored at 2-8° C.
  - 2. Testing should be performed within 72 hours of collection.

### IV. REAGENT

- A. NERL® Blood Bank Saline
  - 1. Unopened stability: marked expiration date
  - 2. Opened stability: 30 days
- B. Monoclonal Anti-A, Anti-B, and Monoclonal Blend Anti-D
  - 1. Stored 1-10° C
  - 2. Stable through marked expiration
- C. Anti- IgG, Monospecific Coombs
  - 1. Stored 1-10° C
  - 2. Stable through marked expiration

### V. INSTRUMENTATION/EQUIPMENT

- A. 12x75 mm Disposable Glass Tubes
- B. Centrifuge
- C. Automatic Cell Washer
- D. 3.5 mL Plastic Transfer Pipettes

### VI. QUALITY CONTROL

- A. Quality control must be done on all reagents once per day on day of use.
- B. CorQC Reagent Cells.
  - 1. Stored at 1-10° C.

2. Stable through marked expiration.
- C. Seraclone® Control ABO + Rh.
  1. Stored at 1-10° C.
  2. Stable through marked expiration.
- D. Coombscell-E, IgG coated Red Blood Cells.
  1. Stored at 1-10° C.
  2. Stable through marked expiration.

**VII. PROCEDURE:**

- A. Label a 12 x 75 mm tube with two patient identifiers (may use a small LIS label).
  1. Place several drops of cord cells in tube and wash by hand 3 times (each washing consists of filling tube with saline and spinning for 1 minute).
  2. Decant completely after third wash and make your 3-5% cell suspension with the remaining washed cells.
- B. Place 1 drop of appropriate Anti-sera (Anti-A, Anti-B, Anti-D and ABO+Rh Control) to properly labeled tubes. Label another tube as DAT. Label all tubes with first and last initial of patient being tested. (Lengthen the minimum letters to differentiate patients with the same initials, if necessary).
- C. Place 1 drop of patient's 3-5% cell suspension in all labeled tubes.
- D. The DAT labeled tube can now be placed in an automatic cell washer for 3 more wash cycles or washed by hand 3 additional times.
- E. Shake all other tubes to mix.
- F. Centrifuge the typing tubes for the optimum spin time determined by the centrifuge calibration (10-30 seconds) at 3400 rpm (approximately 900-1000 rcf).
- G. Gently resuspend cells completely and examine immediately for agglutination. (Use of an optical aid may facilitate detection of weak positive reactions).
- H. Grade and record results in LIS for Type and Rh. The Rh control must be added on in Sunquest in Blood Order Processing – BOP: using the little c key. (See UPPK BB-0606.01 Keyboard attachment).
- I. If the Anti-D tube is negative at immediate spin, the Du or weak D must be carried out by incubating the Anti-D and ABO+Rh control tubes 15 minutes. Then wash 3 times (automatic cell washer may be used), add Anti-IgG monoclonal coombs, mix and centrifuge for the optimum spin time. Read results macro and microscopically. If the Anti-D tube is still negative, add Coombscell-E, now agglutinated, the negative result is valid. The Du and Du control must be added on in Sunquest: using the ' key-to the left of the 1 on the keyboard (see UPPK BB-0606.01 keyboard attachment).
- J. After DAT tube has completed 3 additional washes, decant completely, if not already decanted in automatic cell washer. Add 2 drops of IgG Monospecific Coombs,ox/
- K. Centrifuge for optimum time at 3400 rpm/
- L. Examine for agglutination both macro and microscopically. Record results in Sunquest-BOP.
- M. Add 1 drop of Coombscell-E control cells to any DAT tubes with negative results.
- N. Centrifuge for optimum spin time, read and record results. If the cells are now

agglutinated, the negative result is valid.

**O. Record reactions and interpretations of all testing in Sunquest-BOP.**

**VIII. INTERPRETATION:**

Anti-A	Anti-B	Group Interpretation
NEG	NEG	O
POS	NEG	A
NEG	POS	B
POS	POS	AB

Anti-D	ABO+Rh Control	Interpretation
POS	NEG	D POSITIVE
NEG	NEG	Test for Weak D Antigen
POS	POS	Invalid Result

**IX. REPORTING RESULTS:**

**A. Sunquest Order codes:**

1. CORD (Both ABO+RH and DAT cord profile)
2. ABORH (if just an ABO+Rh type is wanted)
3. DATSCN (if just a DAT is wanted)

**B. For Positive DAT:**

1. If the baby's ABO type is incompatible with the mother's ABO type, AND the mother has a negative antibody screen: Report the POSITIVE DAT with a comment "Positive DAT is probably due to maternal ABO antibody".
2. If the baby and mother have the same ABO type, or baby is Type O: Report the POSITIVE DAT with a comment "The Positive DAT is not due to an ABO incompatibility. It is probably due to an antibody in the mother's serum".
3. An elution may be ordered by the physician and sent out to UPH-Methodist if more information is needed.

**C. For Positive DAT with a Positive Du antigen\*:**

1. If baby's Du reaction is equal in strength to the DAT and ABO+Rh Control reactions: Report baby as Rh NEGATIVE.



2. If baby's Du reaction is greater in strength than the DAT and ABO+Rh Control reactions: Report baby as Rh POSITIVE.

Examples*				
REACTION STRENGTHS			REPORT AS	
Du	CT	DAT	Pt Rh	RhIG
1+	1+	1+	Rh=	no
2+	1+	1+	Rh+	yes

\*If any of these scenarios occur, repeat all testing for reproducibility before reporting.

**X. PROCEDURAL NOTES/PROBLEM SOLVING TIPS:**

- A. In newborns only cell grouping is done, Anti-A and/or Anti-B do not usually appear until the infant is about 3-6 months of age.
- B. Cord blood samples must be washed adequately before testing to remove the Wharton's Jelly which can cause false-positive results.
- C. False negative results of Rh typing may occur when baby's red cells are strongly sensitized with Rh antibody from the mother. In such cases, the DAT will be positive. An elution will reveal Anti-D. The baby should be considered Rh positive.
- D. Babies born to mothers that received ante-partum RhIG may have a positive DAT.


**XI. REFERENCES:**

- A. AABB Technical Manual, 19<sup>th</sup> Edition, 2017.
- B. Bio-Rad Medical Diagnostics GmbH, Blood Grouping Reagent insert, 186241/12, Rev. 03/2017.
- C. Bio-Rad Medical Diagnostics GmbH, Anti-Human Globulin (Anti-IgG) insert, 187822/13, Rev. 08/2014.

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