

Cord Blood Evaluation and Testing Standard Operating Procedure (SOP) - Template

Canadian Obstetrical and Pediatric Transfusion Network recommendations and Prenatal Screening Ontario Fetal *RHD*/Blood Group Genotyping

The Canadian Obstetrical Pediatric Transfusion Network (COPTN) is a sub-committee of the Canadian Society of Transfusion Medicine (CSTM) ([Canadian Obstetrical and Pediatric Transfusion Network - CSTM/SCMT](#)). It was founded in 2017, and its mandate is to assess, analyze and strive to implement best practices in pediatric and obstetrical transfusion practice in Canada.

Prenatal Screening Ontario (PSO) is funded by the Government of Ontario to coordinate the operations of prenatal screening services in Ontario, supporting a cohesive and high-quality screening system. They are Ontario's resources for pregnant individuals, families and health-care professionals looking for information and updates related to prenatal screening ([prenatalscreeningontario.ca](#)). PSO, in partnership with Canadian Blood Services (CBS) and Sinai Health (SH) laboratories, is introducing non-invasive fetal blood group genotyping (FBGG) for pregnant individuals living in Ontario in the fall of 2026.

Two new publicly funded tests will be available in Ontario that will alter the prenatal and postnatal care pathway for RhD negative pregnancies and for those with allo-antibodies to RhD, RhC, Rhc, RhE and Kell (K) antigens. The availability of these new tests will change the required steps in evaluation of RhD immune globulin (RhIG) eligibility and in the testing of cord specimens for alloimmunized pregnancies.

Test Name	Fetal <i>RHD</i> Screening Test	Alloimmunized Fetal Blood Group Genotyping (Allo-FBGG) Test
Purpose	Identifies the fetal RhD status in RhD-negative pregnancies	Determines the fetal antigen status in pregnancies with anti-D, anti-C, anti-c, anti-E, and/or anti-K antibodies
Benefit	Ensures RhIG is administered, only when needed	Supports management of pregnancies at risk for hemolytic disease of the fetus and newborn (HDFN)
Eligibility	RhD negative Singleton pregnancy Has not developed immune anti-D Does not have a known weak D phenotype or <i>RHD</i> variant	Positive for anti-D, -C, -c, -E, and/or -K Singleton and multiple pregnancies
Testing Site	Canadian Blood Services Fetal <i>RHD</i> Screening Laboratory	Sinai Health: Diagnostic Medical Genetics Laboratory

This document will provide suggested updates to the evaluation of cord blood specimens that incorporate review of FBGG results when recommending RhIG.



In the transition period immediately following test introduction, old strategies for evaluation as well as new procedures must be maintained as, initially, not all pregnant individuals will be tested according to the new care pathway or using the new tests.

Laboratories will require access to a provincial clinical viewer (i.e., Connecting Ontario or ClinicalConnect) or the Ontario Laboratory Information System (OLIS) to view these test results. (See [Viewing Fetal RhD Screening and Alloimmunized Fetal Blood Group Genotyping Results](#))

The following information should be included in a Transfusion Medicine Laboratory (TML) cord blood SOP to ensure that your hospital site is performing cord evaluation according to CAN/CSA Z902, CSTM and ACD standards as well as best practice recommendations from COPTN. This document is a template that hospitals can adapt to their current processes to ensure they are aligned with the updated changes from COPTN and FBGG test implementation. Language is generic, and information is true and accurate to standards at time of publication. The information is supportive and does not replace sound judgment or hospital specific policies/requirements. Neither ORBCoN nor PSO are liable for patient specific outcomes.

Principle

Rationale for testing cord blood in the TML:

1. Cord blood testing allows determination of RhD status of the neonate and guides interpretation of birthing client's requirement for RhIG (determines birth parents' RhIG eligibility).
2. Cord blood testing determines neonatal cognate antigen status for alloimmunized pregnancies providing information on whether the neonate is likely to be affected by the pregnant individual's alloantibodies.

Scope/Purpose

This section should lay out the hospital's specific scope of who, what, and when, cord specimens should be evaluated and cord testing performed.

The essential components of the scope/purpose are based on requirements/standards and COPTN statements, and should include:

WHO: Neonates shall have a cord specimen drawn when they are born to a birthing client who is/has:

- RhD Negative, RhD indeterminate, RhD unknown or have an RhD weak type/variant known to be at risk to develop anti-D.
- A history of alloimmunization with a clinically significant antibody.
- A current positive antibody screen.
- A history of HDFN mediated hyperbilirubinemia requiring transfusion of red blood cells (RBC) (intrauterine or previous neonatal exchange) or intravenous immune globulin (IVIG).
- Known risk factors for non-immune neonatal jaundice or anemia (e.g., Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency)

It should be specified in the hospital cord blood policy that if a neonate is born to a birthing client who meets these criteria and does not have a cord blood specimen submitted for testing, that a peripheral specimen



(capillary or venous) is an acceptable alternative. This is especially true for any neonate when the treating clinician predicts an imminent RBC transfusion. Cord blood is **NOT** acceptable for pre-transfusion testing or determination of compatibility.

In certain circumstances, for example stillbirths, a cord or peripheral specimen may not be collected. RhIG should be given immediately or prior to discharge if birthing client would be eligible for RhIG in a live birth.

After the implementation of Fetal Blood Group Genotyping, eligible birthing client's records should be evaluated for presence of Fetal Blood Group Genotyping results. (See [Viewing Fetal RHD Screening and Alloimmunized Fetal Blood Group Genotyping Results](#)). These results are specific to the current pregnancy and the estimated date of delivery (EDD) in the report should be reviewed to ensure the correct results are evaluated.

- If Fetal *RHD* screening results are present and **predict that fetus/newborn is RhD negative**, then the clinician may decide to omit post-delivery infusion of RhIG in the current pregnancy.
- If Fetal *RHD* screening results are present and predict that the fetus/newborn is RhD positive, then RhIG may be issued prior to the completion of cord testing in the current pregnancy.

Cord specimen testing will continue to be performed, until instructed to discontinue testing by PSO/CBS/SH.

What – The following table outlines recommendations of laboratory testing for cord blood specimens.

Test Name	Cord Blood Sample Commonly Recommended (Yes/No)
Pre Transfusion Tests	
ABO	Yes
RhD	Yes
Crossmatch	No
Immunochemistry Investigations	
Weak D (indirect antiglobulin test for RhD)	Yes
DAT	Yes
Euate	Yes
Antibody Screen/ID	No
RBC antigen phenotyping	Yes
Hemolysis Investigations	
Bilirubin	No* /**
G6PD screen/assay	No*
CBC	No*
Reticulocyte Count	No
Blood Film	No

*Only if testing is validated on cord specimens.

** [Canadian Pediatric Society Guidelines](#) recommendation #3 states a pre-discharge total serum bilirubin (TSB) or transcutaneous bilirubin (TcB) is performed in all newborns at a minimum of 12 hours post birth.



When – Cord blood specimens should be received from any clinician (e.g., physician, midwife, nurse practitioner) caring for birthing clients in a hospital or home setting. The laboratory has the ability to set laboratory testing policies that align with [Choosing Wisely](#) and [COPTN's perinatal statements](#) in coordination with appropriate clinical care teams. Testing should be performed if the birthing client meets any of the scenarios for testing. If testing is determined to not be required, cord blood should not be discarded, allowing the clinician to make a request for testing if symptoms of jaundice/HDFN are present within the days following birth. A laboratory policy outlining the length of storage should be established. When testing a cord specimen that has been stored for a prolonged period, reagent manufacturers' recommendations should be followed regarding valid specimen age, and appropriate controls should always be used.

Specimen

EDTA (lavender or pink) or Red/Light Red top vacutainer tube

Min/Max volume according to your laboratory policy

Label requirements – Cord specimen must be labeled with only the neonates' information as specimen identification. Birthing client's information must be submitted with the cord specimen for accurate record correlation but should not be included on the specimen label.

Safety

All laboratory specimens shall be treated with appropriate universal precautions.

Follow all manufacturers' instructions for the safe handling and use of equipment, to reduce risk of contamination, deterioration or operator harm.

Procedure

1.0 Receipt of sample and determination of required testing

- 1.1. Confirm cord specimen is labelled with neonate information as specimen identification.
 - a. If cord specimen is labelled with birthing client information as specimen identification, then reject specimen and request peripheral specimen.
 - b. If neonate is from multiple birth, then a unique identifier to each neonate must be included as specimen identification.
 - c. If cord specimen is submitted from a stillbirth, your hospital may have different labelling requirements for this type of cord specimen.
- 1.2. Confirm birthing client information is linked to the cord specimen. This should include, at minimum, name and Medical Record Number (MRN) of birthing client. This information can be used to perform a history check on the birthing client. Relevant information to check should include: ABO/RhD, antibody screen, Fetal *RHD* screening and/or Allo-FBGG results from testing during pregnancy, and transfusion history.
 - a. Determination of ABO/RhD and antibody screen results may only be available from specimen tested between 8- and 14-week gestation. A method to access this information if performed by a



community laboratory must be available (i.e., provincial clinical viewer, or hard copy) or a new specimen should be requested.

- b. If no ABO/RhD or antibody screen results from current pregnancy are available, contact clinical care area and request Group and Screen testing be performed on birthing client.

1.3. Birthing client's history check will aid in determining appropriate cord specimen testing. Charts [Birthing Client RhD Positive](#) and [Birthing Client RhD Negative/Indeterminate/Unknown](#) guide minimum cord testing required in each scenario.

Birthing client RhD Positive				
Birthing client's antibody screen (in date specimen or specimen within this pregnancy)	History of clinically significant antibody	Fetal RHD screening result	Alloimmunized Fetal Blood Group Genotyping results (D, C, c, E, K)	MINIMUM Cord Testing Required
Negative	None	N/A	N/A	None *
Positive	Yes	N/A	N/A, Inconclusive, Test Failed, or no result	DAT, phenotype for cognate antigen **
			Antigen Positive	
			Antigen Negative	
Negative	Yes (no longer detected)	N/A	N/A, Inconclusive, Test Failed, or no result	DAT, phenotype for cognate antigen **
			Antigen Positive	
			Antigen Negative	

N/A = Not Applicable

*Hold sample for hospital determined retention for request for testing from treating clinician when required.

** If the birthing client has a clinically significant antibody(ies) associated with HDFN (see [Interpretations](#)), antigen phenotyping must be performed on the cord specimen. Specimen may need to be referred out for antigen phenotyping. If birthing client's antibody is not associated with HDFN, antigen phenotyping is not necessarily required.



Birthing Client Rh D Negative / Indeterminate / Unknown				
Birthing client's antibody screen (in date specimen or specimen within this pregnancy)	History of clinically significant antibody	Fetal <i>RHD</i> screening result*	Alloimmunized Fetal Blood Group Genotyping results (D, C, c, E, K)	MINIMUM Cord Testing Required
Negative / Unknown	None	Not done, Inconclusive, or Invalid,	N/A	ABO/RhD
		RhD Positive		
		RhD Negative		ABO/RhD
Positive due to recent RhIG only**	None	Not done, Inconclusive, or Invalid	N/A	ABO/RhD, DAT
		RhD Positive		
		RhD Negative		ABO/RhD, DAT
Positive	Yes	Not done, Inconclusive, or Invalid	N/A, Inconclusive, Test Failed, or no result	ABO/RhD, DAT, phenotype for cognate antigen ***
		RhD Positive	Antigen Positive	
		RhD Negative	Antigen Negative	
Negative	Yes (no longer detected)	Not done, Inconclusive, or Invalid	N/A, Inconclusive, Test Failed, or no result	ABO/RhD, DAT, phenotype for cognate antigen ***
		RhD Positive	Antigen Positive	
		RhD Negative	Antigen Negative	

N/A = Not Applicable

* Pregnant individuals with immune anti-D are not eligible for Fetal *RHD* screening but **are** eligible for Allo-FBGG testing.

** Anti-D due to RhIG is not considered a clinically significant antibody. This must be differentiated from an immune anti-D.

Differentiation may include but not limited to: confirming a recent dose (within previous 3 months) of RhIG, antibody strength not greater than 2+, mini panel to rule out all other clinically significant antibodies. Medical director consultation may be required.

*** If the birthing client has a clinically significant antibody(ies) associated with HDFN (see [Interpretations](#)), antigen phenotyping must be performed on the cord specimen. Specimen may need to be referred out for antigen phenotyping. If birthing client's antibody is not associated with HDFN, antigen phenotyping is not necessarily required.

1.4. If testing is not required, report as testing not indicated and hold specimen for a determined length of time based on laboratory policy.

- a. Clinicians may request testing if signs of jaundice or HDFN are present in neonate after laboratory determines testing not required with table above. A comment to indicate testing "At Clinician's Request" should be included.

1.5. If testing is appropriate, proceed to test the specimen.



2.0 Specimen Testing

- 2.1 Perform ABO/RhD as appropriate with laboratory testing methodology for cord specimens (see [Interpretations](#)).
- 2.2 RhD negative cord of an RhD negative birthing client must have weak D testing performed.
- For valid weak D interpretations, a DAT on cord specimen must be negative.
 - For sites that refer out weak D testing, consideration may be given to recommend RhIG while waiting for test results, depending on turnaround time of testing and discharge of birthing client.
 - See [interpretations](#) chart for determination of recommending RhIG.
- 2.3 RhD positive cord results of an RhD negative client, RhIG must be recommended and fetomaternal hemorrhage (FMH) test sent (see [interpretations](#) chart).
- 2.4 If Fetal *RHD* screening was performed, ensure cord specimen testing corresponds with Fetal *RHD* screening result.

If discrepant, then report discrepancy to Canadian Blood Services Fetal *RHD* screening Laboratory:



905-494-8132



fetalRHD@blood.ca

- 2.5 Perform DAT as appropriate with laboratory testing methodology for cord specimens (see [interpretations](#)).
- Cord DAT should consist of anti-IgG only
 - Any cord specimen with a positive DAT cannot have weak D testing unless further treatment of cord red cells is performed to dissociate the bound IgG (e.g., EGA).
 - Any cord specimen with a positive DAT cannot have antigen phenotyping performed using an indirect testing method (use of anti-IgG in procedure) unless further treatment of cord red cells is performed to dissociate the bound IgG (e.g., EGA).
 - Note:** almost all phenotyping reagents for antigens implicated in HDFN are now available as direct agglutinating reagents.
 - All positive DATs on cord specimens must be evaluated to explain the reason for the positive DAT (e.g., ABO incompatibility, positive antigen phenotyping corresponding to birthing client's antibody, RhD positive cord in birthing client who received prophylactic [28 week] RhIG) (see [Positive DAT interpretation](#)).
 - Eluates on cord specimens are rarely required but may be indicated when there is no plausible explanation for positive DAT or when more than one antibody could be contributing to the positive DAT. Consult transfusion medicine (TM) physician or immunohematology expert as required.
 - In cases of unexplained severe jaundice in a newborn, a negative DAT may require an eluate or additional testing. Consult TM physician or immunohematology expert as required.
- 2.6 Perform antigen phenotyping according to antisera manufacturers' recommendation.
- All positive antigen phenotyping corresponding to birthing clients clinically significant antibody associated with HDFN, must be reported to the most responsible clinician (see [Interpretations](#) chart).



b. For antigens known to have weaker expression in cord specimens, see [interpretations](#).

2.7 If Allo-FBGG was performed, ensure cord antigen phenotype testing corresponds with fetal blood group genotyping result.

If discrepant, then report discrepancy to Sinai Health: Diagnostic Medical Genetics Laboratory:



416-586-4800 x5974



FBGG.MSH@sinaihealth.ca

2.8 Additional testing may be required based on results obtained on cord specimen. Refer to the [Cord Blood Testing Algorithm](#).

Interpretations

ABO/RhD and DAT interpretation on cord specimens*/**

Anti-A	Anti-B	Anti-A,B	Control (Inert)	ABO Blood Group Interpretation
1+ to 4+	0	1+ to 4+	0	A
0	1+ to 4+	1+ to 4+	0	B
1+ to 4+	1+ to 4+	1+ to 4+	0	AB
0	0	0	0	O
Any	Any	Any	Wk to 4+	Invalid
Anti-D	Weak D	Control (Inert)		Rh Blood Group Interpretation
0	Negative	0		RhD Negative
	Positive			RhD Positive
1+ to 4+	N/A	0		
Any		Wk to 4+		Invalid
DAT (IgG only)		Control (Inert)		DAT Interpretation
0		0		DAT Negative
Wk+ to 4+		0		DAT Positive
Any		Wk to 4+		Invalid

Wk = Weak, microscopic

*Wharton's jelly may interfere with cord blood testing resulting in false positive results. A mechanism to limit Wharton's jelly interference should be included as part of testing.

**Neonatal ABO antigens may not be fully developed at birth and may show weak or mixed field reactions. If mixed field reactions are noted, consult with clinical care area to ensure that an intrauterine transfusion has not occurred.



Recommending RhIG and Fetomaternal Hemorrhage (FMH) Testing

The following chart provides guidance on when to recommend RhIG to the birthing client and FMH testing.

Birthing client RhD status	Fetal RHD screening result	Cord RhD result	Cord Weak D result	FMH testing required?	RhIG recommended?
Positive	N/A	Positive	N/A	No	No
Positive	N/A	Negative	N/A	No	No
Negative / Indeterminate	Positive	Positive	N/A	Yes	Yes
Negative / Indeterminate	Not done, Inconclusive, Invalid	Positive / indeterminate	N/A	Yes	Yes
Negative / Indeterminate	Negative	Negative	Negative	No	No
			Positive	Yes	Yes
Negative / Indeterminate	Not done, Inconclusive, Invalid	Negative	Negative	No	No
			Positive	Yes	Yes

N/A = Not Applicable

- If Fetal RHD screening indicates that the birthing client is eligible for RhIG, RhIG should not be held up from issuing to clinical care area pending confirmation of cord testing.
- First dose of RhIG should not be delayed if referred out or FMH test cannot be performed prior to the birthing client's discharge. First dose of RhIG should be given within 72hrs of delivery. (RhIG may be given up to 28 days post-delivery)
- Indeterminate RhD result of the cord/neonate should be treated as RhD positive, therefore, birthing client requires FMH test and RhIG should be recommended.
- A birthing client with known immune anti-D does not require post-partum RhIG.
- When FMH test is not required as per above chart, it could still be requested by clinical team as part of an investigation for fetal/neonatal anemia.

Antibodies associated with HDFN

The following red blood cell antibodies have been identified as causing HDFN. This list is comprised of those antigens which are common on routine commercial cells used for antibody identification. There may be additional antigens that are low in prevalence, or the antigens do not routinely show up on antigram panels used in antibody investigations that are not included in this list. If the pregnant individual's antibody screen is positive, but panel is negative, referral to antibody reference lab is recommended to rule out a clinically significant antibody to a low prevalence antigen. Similarly, if there is a positive neonatal or cord blood DAT, with or without evidence of anemia or jaundice, investigation of the pregnant individual's specimen for an antibody to a low prevalence antigen is recommended prior to any subsequent pregnancies. Antibodies with reactivity to multiple cells or all panel cells that cannot be identified should be investigated for antibodies to a high prevalence antigen and correlated with risk for HDFN.

Consultation with TM physician or immunohematology subject matter expert may be required.



Association	Severity of HDFN Blood Group System (antigen)	
	Mild*	Mild to Severe
Commonly associated with HDFN	ABO (A, B) – HDN only Rh (E**, e, C**)	Rh (D**, c**, G) Kell (K**, k)
Rarely associated with HDFN	Duffy (Fy ^b , Fy ³) Kidd (Jk ^b , Jk ³)	Kell (Kp ^a , Kp ^b [moderate], Js ^a , Js ^b) Duffy (Fy ^a) Kidd (Jk ^a) MNS (S, s, U, rarely M) Diego (Di ^a , Di ^b , Wr ^a)
Not associated with HDFN	ABO (A ₁) P (P ₁) Lewis (Le ^a , Le ^b) MNS (N) Xg ^a Chido/Rodgers Lutheran (Lu ^a , Lu ^b)	

* Rare cases may be severe.

** Fetal blood group genotyping (FBGG) testing is available.

Cord Phenotyping

Weak antigen expressions in cord specimens are common for the following antigens that are associated with HDFN; A, B, Fy³. Interpret negative results with caution.

Results Reporting

Cord ABO/RhD, DAT and antigen phenotyping results must be reported to the clinician for RhIG ordering or for further work up DAT/antigen positive neonates. These results should be reported in the Laboratory Information System (LIS) as a comment on the cord specimen, the peripheral neonatal specimen and/or on the birthing client's MRN. Depending on laboratory policy a phone call to the clinician may also be required.

Discrepancies between FBGG results and serological cord testing should be reported to the testing laboratory (CBS-Fetal RHD Screening, SH – Allo-FBGG)

Antigen Phenotyping interpretation

When cord antigen phenotyping is positive for the antigen corresponding to the birthing clients clinically significant antibody, a comment such as the following should be on the final report:

The birthing client has a clinically significant antibody (anti-XX) capable of causing HDFN. Cord red blood cells type as antigen (XX) positive/negative (please select).

(If antigen positive) This neonate is at risk for HDN, and monitoring of hemoglobin and bilirubin is advised (according to Canadian Pediatric Society (CPS) hyperbilirubinemia guidelines)



Positive DAT interpretation

Positive DAT results on any cord specimen, should have a comment communicating to clinical team the reason for the positive DAT. The following are examples of canned text comments that may be used. In certain situations, a combination of the following may cause a positive DAT (e.g., ABO incompatibility and passive anti-D in an RhD positive cord). All combinations should be reported.

ABO incompatibility (e.g., birthing client is group O and cord specimen types as non-group O):

Cord red blood cells ABO type as group A, B, or AB (please select)

Probable ABO incompatibility between the birthing client and neonate. Positive cord DAT is most likely due to birthing client anti-A/B (please select). In the setting of jaundice or anemia additional testing/consultation could be considered.

Passive Anti-D and cord RhD Positive:

The birthing client's plasma contains passive Anti-D. Positive cord DAT is most likely due to recent injection of RhIG (insert date of RhIG injection). In the setting of unexpected jaundice or anemia additional testing/consultation could be considered.

Clinically significant antibody and cord antigen positive:

The birthing client's plasma contains a clinically significant antibody (Anti-XX). Cord red blood cells type as positive for the associated antigen, and the positive DAT is most likely due to the (Anti-XX). Monitoring for jaundice and anemia is recommended along with neonatology consultation.

RhIG recommendation and suggested comment

All RhD negative or indeterminate birthing clients shall have recommendations on eligibility for post-partum RhIG. This recommendation may be reported on the cord specimen, neonatal peripheral specimen or reported on the birthing clients MRN. The following is an example of canned text comment that may be used.

It is/is not (please select) recommended that this birthing client receives a post-partum dose of RhIG. Please follow hospital policy for ordering blood products.

FMH test recommendation and suggested comment

All RhD negative or indeterminate birthing clients shall have a FMH test specimen collected prior to first dose of RhIG being administered when cord RhD testing is positive or unknown. This recommendation may be reported on the cord specimen, neonatal peripheral specimen and/or reported on the birthing clients' MRN. The following is an example of canned text comment that may be used.

It is/is not (please select) recommended that this birthing client has a specimen collected for Fetomaternal Hemorrhage (FMH) testing . Based on results of FMH test, additional RhIG doses may be required.

Limitations

1. To ensure all birthing clients eligible for RhIG receive RhIG, TML should not rely solely on specimens submitted for laboratory testing. Laboratories should investigate with their obstetrical/delivery department a mechanism, such as a list of neonates delivered, to ensure no eligible birthing clients are missed.



2. Birthing clients with known immune anti-D do not require post-partum RhIG. If immune vs. passive anti-D is in question, it is appropriate to err on the side of caution and issue a dose of RhIG and reassess immune vs. passive antibody status in 6 months when RhIG should be cleared.
3. The presence of anti-G in birthing clients may mimic anti-D and/or anti-C. If the presence of immune anti-D is ruled out, the birthing client is eligible for RhIG.

References

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Document History

Date	Revisions
2026	New document

