GUELPH GENERAL HOSPITAL

Rh Genotyping – Lessons from the Lab

FOR GHEST - SANDRA BAKKER – SEPTEMBER 16TH 2023

Disclosures

- No financial disclosures
- No speaker disclosure
- NOT an expert!!!

Objectives

1. Identify when RhD discrepancy testing is required
2. Discuss past practice of RhD discrepancy testing at GGH
3. Discuss *briefly* the RhD antigen, weak D and partial D antigens
4. Discuss indications for RHD genotyping
5. Summarize the reasons for making changes to our process
6. List current practice of RhD discrepancy testing at GGH


When is Rh Discrepancy
Testing Required?

When is RhD Discrepancy Testing Required?

1. Discrepancy between previous Rh(D) blood grouping and current Rh(D) blood grouping
2. Weak reaction (<3+) with anti-D reagent
3. Unexplained (i.e. not due to transfusion) mixed field reaction with anti-D reagent
4. Unexpected positive reaction with negative control test

Past Practice at GGH

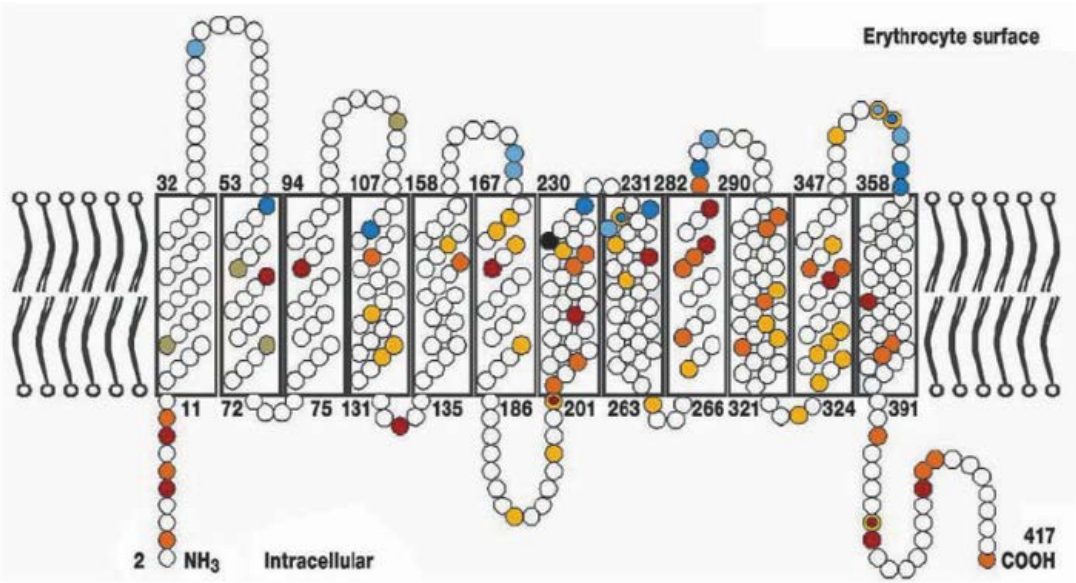
GGH Rh Discrepancy Testing – PAST-1

- Test with two types of anti-D (plus negative control)
  - First with Immucor and then Biorad reagents
    - Reagent manufacturer & lot # recorded in house only
- Algorithm depending on type of discrepancy
  - Weak test results with anti-D reagent
  - Mixed field with anti-D reagent
  - Rh type on current sample different from previous results

GGH Rh Discrepancy Testing – PAST-2

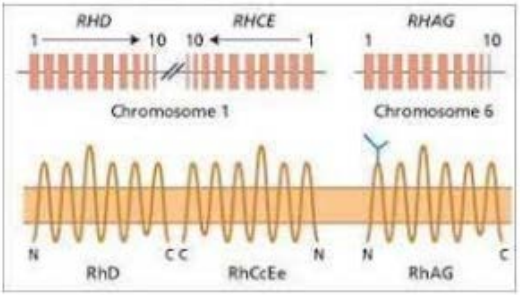
- Follow manufacturer's instructions of use of antisera, which over the years has included:
  - Incubate at 37C for 30 minutes
  - Incubate at RT for 15 minutes
  - Perform Weak D (IAT) testing (many years ago included non donor/newborn)
- Report at Rh(D) Positive if 3+ reaction after incubation
- Decide if patient treated as Rh(D) positive or Rh(D) negative
- When genotyping became available, send females <45 years of age for Rh(D) genotyping
- Fill out CBS requisition, often ticking UNKNOWN box for patient ethnicity

RHD Antigen


Erythrocyte surface
32
53
94
107
158
167
230
231
282
290
347
358
11
72
75
131
135
186
201
263
266
321
324
391
2
NH_3
Intracellular
417
COOH

The Rh Blood Group System

- Rh Blood Group system made up of 56 different antigens
- Encoded by two genes - RHD and RHCE genes



https://www.isbtweb.org/isbt-working-parties/rcibgt.html

Reid ME et al. The Blood Group Antigen Facts Book. 2012

D Antigen or RH1

- Rh(D) antigen is RH1 – what makes the blood group Rh(D) Positive or Rh(D) Negative
- Rh(D) positive expectation varies ethnicity
  - Caucasian = 85% Rh(D) positive
  - Blacks = 92% Rh(D) positive
  - Asians and Native Americans = high prevalence Ag

Caucasians	Blacks	Asians	Native Americans
85%	92%	99%	99%

Reid ME et al. The Blood Group Antigens Facts Book. 2012

D Antigen (RH1)

- Rh(D) negative phenotype
  - Total absence of D protein on the RBC membrane
  - In Caucasians: caused by deletion of the RHD gene
  - In Blacks: frequently caused by inactive RHD gene
- Expressed on cord and adult RBCs
- Highly immunogenic
  - Mild to severe HDFN
  - Mild to severe/immediate or delayed HTR

Reid ME et al. The Blood Group Antigens Facts Book. 2012

Weak D Phenotype

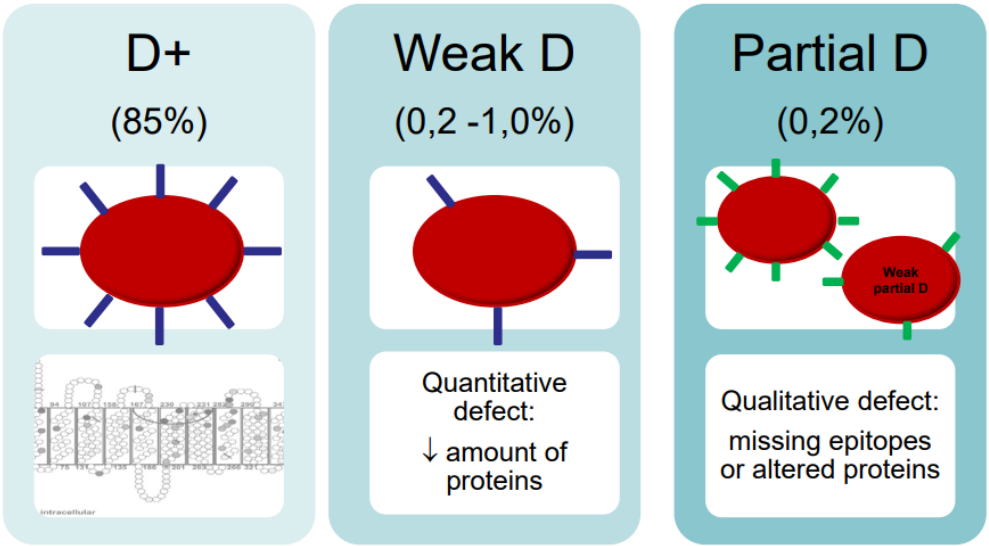
- **Quantitative** polymorphism
- Codes for decreased amount of the RhD antigen
- Results in decreased antigen density on the red cell membrane
- Occurs in 0.2 – 1.0 % of population
- Normal D antigen – just less of it
- Individuals who are partial D and exposed to a normal D antigen = no foreign protein detected – no immune anti-D produced

Partial D phenotype

- **Qualitative** polymorphism
- Missing epitopes or altered proteins
- Less common than Weak D
- Occurs in 0.2%
- Individuals who are partial D and exposed to a normal D antigen can produce an immune anti-D to the unrecognized part of the D antigen (foreign)

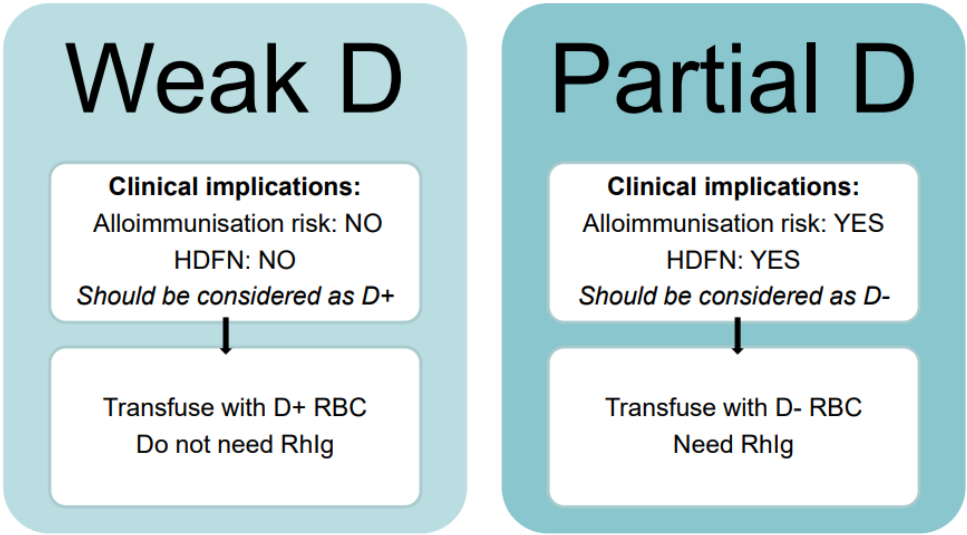
Robitaille, Nancy. 2022

Weak D vs Partial D



Robitaille, Nancy. 2022

Clinical Implications

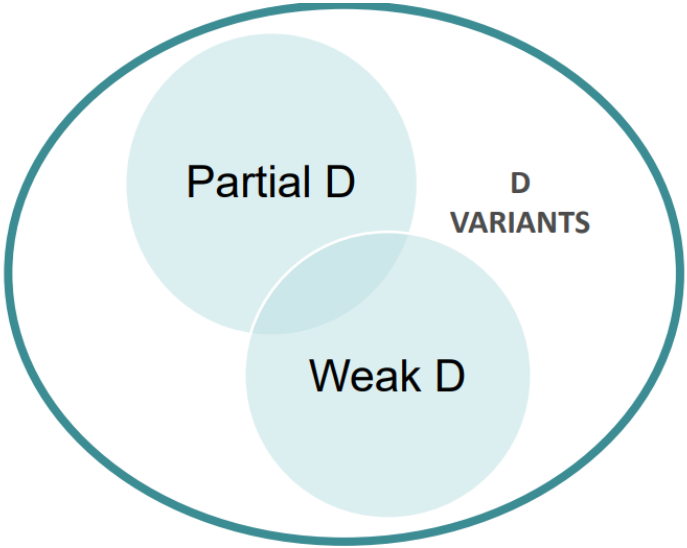


Robitaille, Nancy. 2022

Weak D vs Partial D – Serological Tests

- Serological test results depends on many factors
- Weaker than expected results seen
  - 2+ or weaker reaction with anti-D reagent
  - Possibly even 3+ can be an indication
- Difference between testing platforms or reagents
  - Gel is more sensitive than tube
  - Different anti-D reagents (monoclonal or polyclonal) can give varying results
- Apparent Rh Positive patient who presents with an immune anti-D or appear to have an auto-anti-D

D Variants, what??



Robitaille, Nancy. 2022

RHD Genotyping

Recommendations from Transfusion - 1

- Perform RHD genotyping whenever discordant RHD typing results and/or serologic weak D phenotype is detected in a female with childbearing potential.
- Persons with weak D type 1, 2 or 3 should be managed as Rh+
  - Fewer unnecessary injections of RhIG
  - Increased availability of Rh negative RBCs for transfusion
- In order to facilitate implementation:
  - Large-scale testing
  - Reference laboratories performing RBC genotyping should offer affordable tiered services

Transfusion 2015; 55:680-689

Recommendations from Transfusion - 2

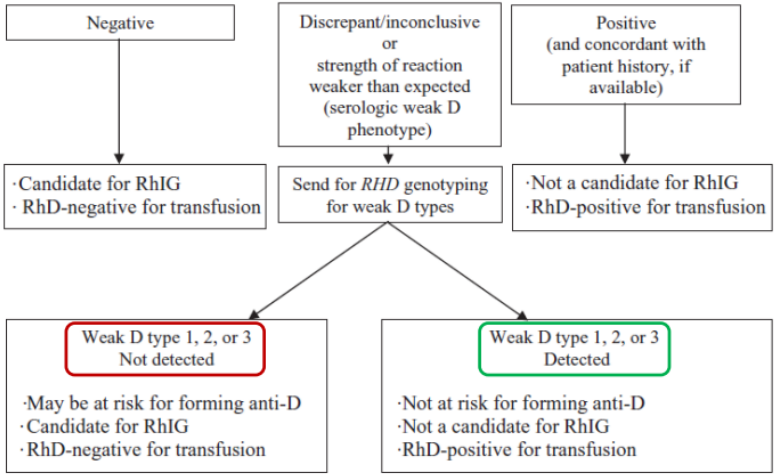


Fig. 3. Algorithm for resolving serologic weak D phenotype test results by *RHD* genotyping to determine candidacy for RhIG and RhD type for transfusions.

Transfusion 2015; 55:680-689

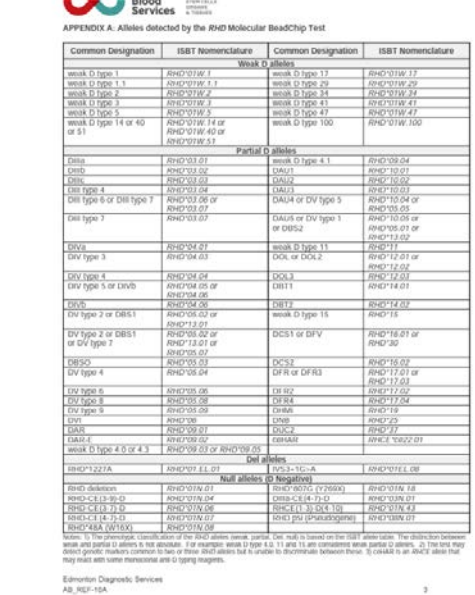
What is RHD Genotyping? - 1

- As per the CBS website:
- PCR based assay for the identification of **many** clinically significant RHD variants (alleles) responsible for the normal and altered/absent expressions of the RhD antigen
- Used to *predict* the RhD status of a patient

What is RHD Genotyping? - 2

- Testing performed by CBS in Edmonton
- Immucor RHD Molecular BeadChip Test
  - Tests for 35 genetic markers to resolve **66** alleles
  - Detects *most common* RHD variants associated with Weak D, partial D, Del, and D negative phenotypes
  - Does not involve sequencing of the RHD gene

Alleles Detected by CBS Testing



Canadian Blood Services

APPENDIX A: Alleles detected by the *RHD* Molecular BeadChip Test

Common Designation	ISBT Nomenclature	Common Designation	ISBT Nomenclature
Weak D alleles			
weak D type 1	*RHD*01W.1*	weak D type 17	*RHD*01W.17*
weak D type 1.1	*RHD*01W.1.1*	weak D type 29	*RHD*01W.29*
weak D type 2	*RHD*01W.2*	weak D type 34	*RHD*01W.34*
weak D type 3	*RHD*01W.3*	weak D type 41	*RHD*01W.41*
weak D type 5	*RHD*01W.5*	weak D type 47	*RHD*01W.47*
weak D type 14 or 40 or 51	*RHD*01W.14* or *RHD*01W.40* or *RHD*01W.51*	weak D type 100	*RHD*01W.100*
Partial D alleles			
DIIIa	*RHD*03.01*	weak D type 4.1	*RHD*09.04*
DIIIb	*RHD*03.02*	DAU1	*RHD*10.01*
DIIIc	*RHD*03.03*	DAU2	*RHD*10.02*
DIII type 4	*RHD*03.04*	DAU3	*RHD*10.03*
DIII type 6 or DIII type 7	*RHD*03.06* or *RHD*03.07*	DAU4 or DV type 5	*RHD*10.04* or *RHD*05.05*
DIII type 7	*RHD*03.07*	DAU5 or DV type 1 or DBS2	*RHD*10.05* or *RHD*05.01* or *RHD*13.02*
DIVa	*RHD*04.01*	weak D type 11	*RHD*11*
DIV type 3	*RHD*04.03*	DOL or DOL2	*RHD*12.01* or *RHD*12.02*
DIV type 4	*RHD*04.04*	DOL3	*RHD*12.03*
DIV type 5 or DIVb	*RHD*04.05* or *RHD*04.06*	DIB11	*RHD*14.01*
DIVb	*RHD*04.06*	DIB12	*RHD*14.02*
DV type 2 or DBS1	*RHD*05.02* or *RHD*13.01*	weak D type 15	*RHD*15*
DV type 2 or DBS1 or DV type 7	*RHD*05.02* or *RHD*13.01* or *RHD*05.07*	DCS1 or DFV	*RHD*16.01* or *RHD*30*
DBSO	*RHD*05.03*	DCS2	*RHD*16.02*
DV type 4	*RHD*05.04*	DFR or DFR3	*RHD*17.01* or *RHD*17.03*
DV type 6	*RHD*05.06*	DFR2	*RHD*17.02*
DV type 8	*RHD*05.08*	DFR4	*RHD*17.04*
DV type 9	*RHD*05.09*	DHM6	*RHD*19*
DVI	*RHD*06*	DNB	*RHD*23*
DAR	*RHD*09.01*	DUC2	*RHD*27*
DAR-I	*RHD*09.02*	DBHAR	*RHCE*06[22.01*
weak D type 4.0 or 4.3	*RHD*09.03* or *RHD*09.05*		
Del alleles			
RHD*1227A	*RHD*01.EL.01*	IVS-3+1G>A	*RHD*01.EL.08*
Null alleles (D Negative)			
RHD deletion	*RHD*01N.01*	RHD*807G (Y269X)	*RHD*01N.18*
RHD-CE-(3-9)-D	*RHD*01N.04*	DIIIa-CE(4-7)-D	*RHD*03N.01*
RHD-CE(3-7)-D	*RHD*07N.06*	RHCE(1-3)-D(4-10)	*RHD*07N.43*
RHD-CE(4-7)-D	*RHD*01N.07*	RHD psi (Pseudogene)	*RHD*08N.01*
RHD*48A (W16X)	*RHD*01N.08*		

Notes: 1) The phenotypic classification of the *RHD* alleles (weak, partial, Del, null) is based on the ISBT allele table. The distinction between weak and partial D alleles is not absolute. For example: weak D type 4.0, 11 and 15 are considered weak partial D alleles. 2) The tins may detect genetic markers common to two or three *RHD* alleles but is unable to discriminate between these. 3) cis-AR is an *RHCE* allele that may react with some monoclonal anti-D typing reagents.

Edmonton Diagnostic Services
AB_REF-10A

RHD Variants – How many are there?

- The RHD gene shows remarkable biodiversity with **several hundred** unique alleles listed in the International Society of Blood Transfusion (ISBT) allele table and described in the literature.
- The number of identified RHD alleles has increased significantly in recent years with advances in sequencing technology and large population-based studies.

RHD Variants – Ethnic Variations

- The distribution of RHD alleles *varies across different ethnic and geographic populations*
- In the context of the ethnically diverse Canadian population, *some variant RHD alleles will not be identified* by a targeted RHD genotyping assay

How are the Results Interpreted?

- CBS performs a careful review of the genetic markers from the testing, the patient clinical history, and lab serologic test results
- Clinical history
  - Clinical indication (prenatal, sickle cell disease, hemoglobinopathy, chronic transfusion recipient)
  - History of stem cell transplant or hematologic neoplasm
  - Recent (<3 months) transfusion history
- Serological results
  - Typing results
  - Tube testing using different anti-D reagents
  - Best to include the manufacturer, lot number, and results of the hospital lab testing

Changes to GGH Practice

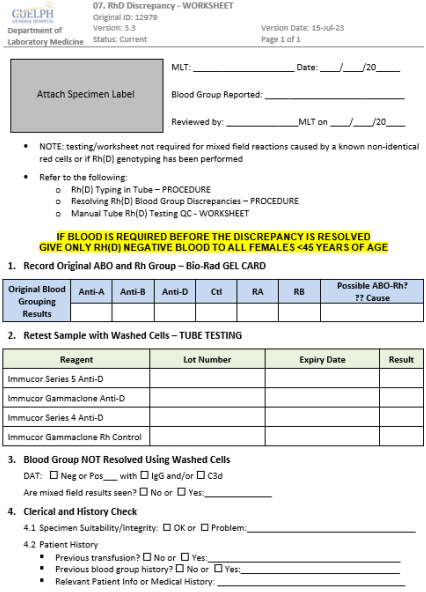
Why did we Change our Process?

- Discussion with Dr. Melanie Bodnar at CBS in Edmonton
- We had a patient sent for genotyping that required further interpretation
- Questions she had - What methods do we use? What were our reactions with our tube testing reagents? What reagents did we use? What is the ethnicity of the patient?
- She explained the process at CBS and what would make the whole testing experience better

Current steps prior to RhD genotyping

1. Tube testing (washed patient cells)
2. Use of three specific antisera
   - Immucor Series 5
   - Immucor Series 4
   - Immucor Gammaclone
3. Lot numbers and reactions sent with genotype request
4. No incubation, Weak D (IAT) testing, or other
5. More accurate patient history (i.e. ethnicity)

Worksheet sent with Requisition



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Department of Laboratory Medicine

07. RhD Discrepancy - WORKSHEET
Original ID: 12979
Version: 3.3
Status: Current
Version Date: 15-Jul-23
Page 1 of 1

Attach Specimen Label

MLT: _____ Date: ____/____/20____

Blood Group Reported: _____

Reviewed by: _____MLT on ____/____/20____

- NOTE: testing/worksheet not required for mixed field reactions caused by a known non-identical red cells or if Rh(D) genotyping has been performed
- Refer to the following:
  - Rh(D) Typing in Tube – PROCEDURE
  - Resolving Rh(D) Blood Group Discrepancies – PROCEDURE
  - Manual Tube Rh(D) Testing QC - WORKSHEET

IF BLOOD IS REQUIRED BEFORE THE DISCREPANCY IS RESOLVED
GIVE ONLY RH(D) NEGATIVE BLOOD TO ALL FEMALES <45 YEARS OF AGE

1. Record Original ABO and Rh Group – Bio-Rad GEL CARD

Original Blood Grouping Results	Anti-A	Anti-B	Anti-D	Ctl	RA	RB	Possible ABO-Rh? ?? Cause

2. Retest Sample with Washed Cells – TUBE TESTING

Reagent	Lot Number	Expiry Date	Result
Immucor Series 5 Anti-D			
Immucor Gammaclone Anti-D			
Immucor Series 4 Anti-D			
Immucor Gammaclone Rh Control			

3. Blood Group NOT Resolved Using Washed Cells

DAT: ☐ Neg or Pos___ with ☐ IgG and/or ☐ C3d

Are mixed field results seen? ☐ No or ☐ Yes:_____

4. Clerical and History Check

4.1 Specimen Suitability/Integrity: ☐ OK or ☐ Problem:_____

4.2 Patient History

- Previous transfusion? ☐ No or ☐ Yes:_____
- Previous blood group history? ☐ No or ☐ Yes:_____
- Relevant Patient Info or Medical History: _____

5. Send a copy of this worksheet with any Rh(D) Genotyping Request

CBS Requisition

- We fill it out completely
- Will call the patient care area to get more information
  - Patient ethnicity
  - Diagnosis or other medical history not found in LIS
- Include a copy of our discrepancy testing results with the completed requisition

Who gets Rh(D) Genotyping at GGH?

The following patients who have weak (less than 3+ or 2+ or less) reactions with *any* anti-D reagent

- Females less than 45 years of age
- Patients expected to be frequently or chronically transfused

  (i.e. sickle cell, thalassemia, or any other reason)
- Patients with really unexpected results (i.e. very weak reactions, varying reactions between reagents, different from previous) might be sent
- Others? Treat as Rh(D) positive

Thanks

- Special thanks to Dr. Melanie Bodnar
- All errors are mine ☺ Questions?

References

- Reid ME et al. The Blood Group Antigen Facts Book. 2012
- Robitaille, Nancy. When should RhD genotyping be performed for pregnant D negative women. Presentation for Choosing Wisely when performing antenatal and postnatal transfusion tests. May 25, 2022. https://transfusionontario.org/when-should-rhd-genotyping-be-performed-for-pregnant-d-negative-women/
- Sandler SG, et al.; It's time to phase in RHD genotyping for patients with a serologic weak D phenotype. Transfusion. 2015 Mar;55(3):680-9. doi: 10.1111/trf.12941. Epub 2014 Dec 1. PMID: 25438646; PMCID: PMC4357540