ORBCoN
Ontario Regional Blood Coordinating Network

Inspiring and facilitating best transfusion practices in Ontario.

When should RhD genotyping be performed for pregnant D negative women?

Nancy Robitaille, MD, FRCPC

Choosing wisely when performing antenatal and postnatal transfusion tests

May 25, 2022

Faculty Disclosure

In compliance with CPD policy, Temerty Faculty of Medicine requires the following disclosures to the session audience

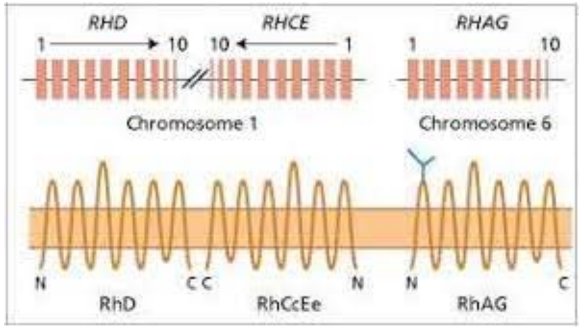
- This program has received no financial external support
- Speaker disclosure: nothing to disclose

Objectives

- Describe differences between weak and partial D
  - Including initial lab testing results that suggest RHD genotyping is required
- Highlight clinical scenarios that warrant ordering a genotype test to verify the subtype of weak D
- Discuss practical aspects of genotype testing
  - At what point in pregnancy test should be ordered, forms to complete (CBS and Héma-Québec), testing platforms, typical turn-around time for results, feasibility to send samples, accessibility of results to all hospitals
- Discuss the need to provide RhIg prophylaxis for bleeds that occur in patients with weakly reactive RhD and genotype results are not available
- Know which subtypes of weak D warrant anti-D prophylaxis and which can be considered RhD positive

Rh Blood Group System

- Number of antigens: 56
  - Comprises polymorphic, high prevalence and low prevalence Ag



- Genes:
  - RHD and RHCE
    - Located on chromosome 1p36.11
    - Each have 10 exons
    - Opposite orientation with 3'ends facing each other
  - RHAG
    - Located on chromosome 6
    - Encodes the Rh-associated glycoprotein (RHAG) which is essential for the expression of Rh Ag

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

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

D Antigen (RH1)

- Occurrence varies according to ethnicity:

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

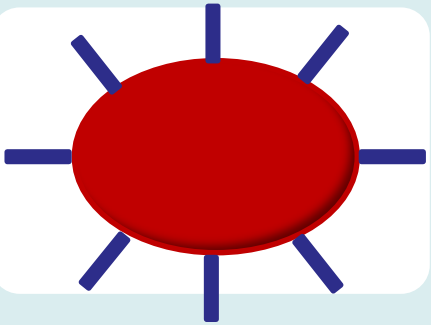
- D- phenotype: total absence of D protein from the RBC membrane:
  - Caucasians: deletion of RHD gene
  - Blacks: inactive RHD gene (RHD pseudogene or RHDψ)
- Expressed on cord and adult RBCs
- Highly immunogenic
  - Mild to severe HDFN
  - Mild to severe/immediate or delayed hemolytic transfusion reaction

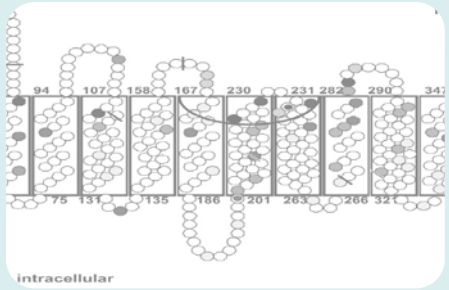
Reid ME et al. The Blood Group Antigen Facts Book. 2012

Weak D vs Partial D

D+

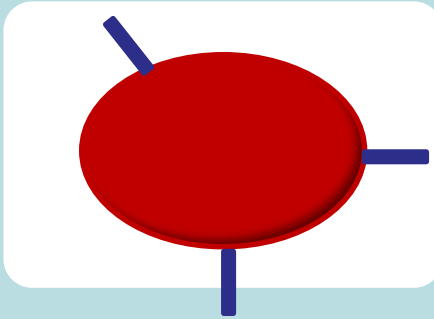
(85%)





Weak D

(0,2 -1,0%)

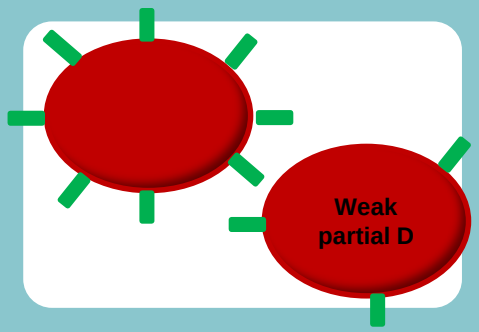


Quantitative defect:

↓ amount of proteins

Partial D

(0,2%)



Qualitative defect:

missing epitopes or altered proteins

Weak D vs partial D: What does it mean in practice?

Weak D

Clinical implications:
Alloimmunisation risk: NO
HDFN: NO
Should be considered as D+

Transfuse with D+ RBC
Do not need RhIg

Partial D

Clinical implications:
Alloimmunisation risk: YES
HDFN: YES
Should be considered as D-

Transfuse with D- RBC
Need RhIg

Weak D vs partial D: What does it mean in the blood bank?

Weak D

D testing:

Weak (≤ 2+) or negative results

Weak D test: stronger rxns

(not recommended routinely)

↓

Strength of the reaction can vary according to the antiserum used, technique used, etc.

Partial D

D testing:

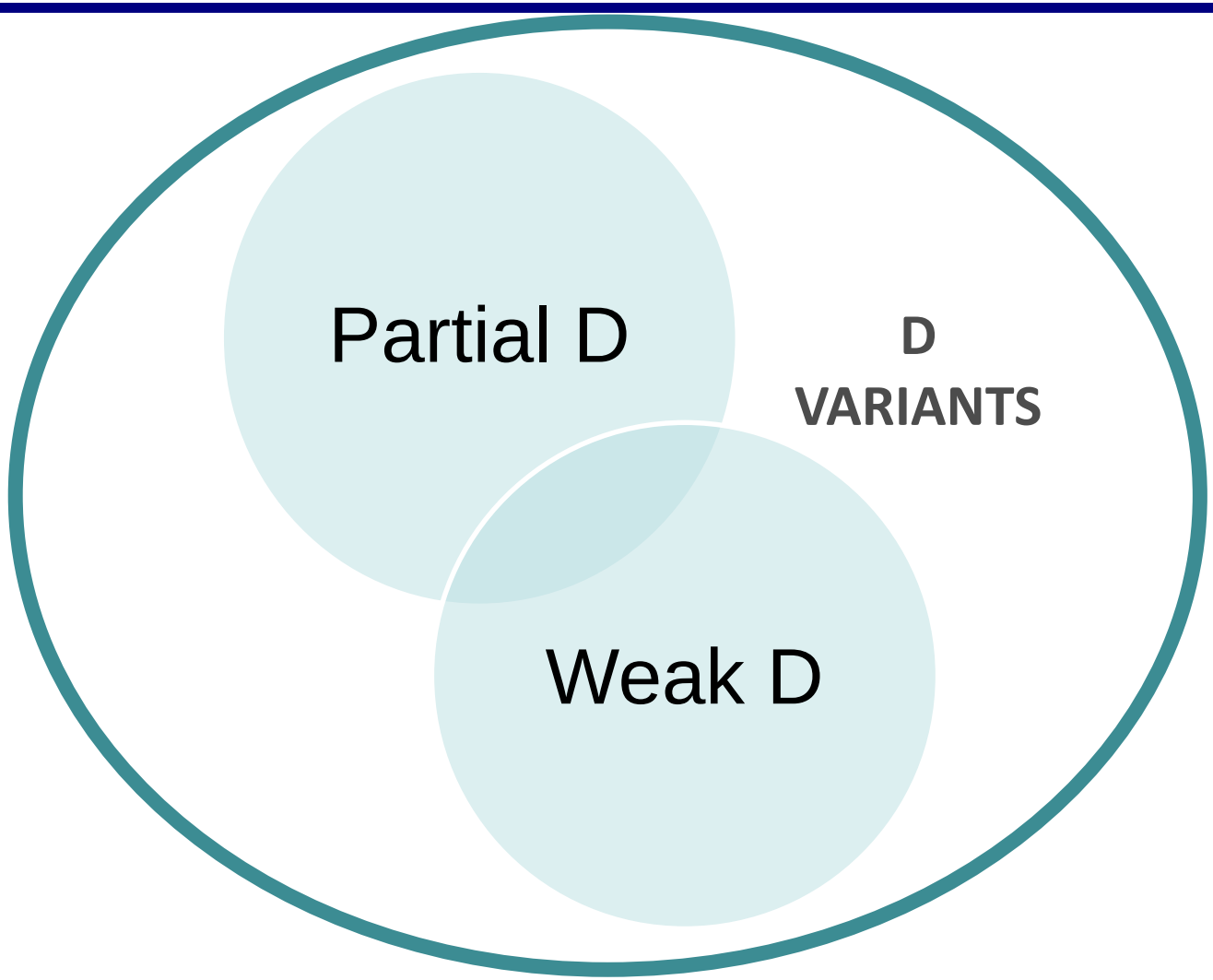
Normal strength reaction, except for partial weak D

D+ recipient who develops anti-D

↓

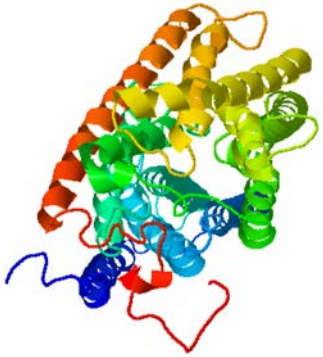
Capacity of an antiserum to detect a partial D is quite variable

What are D variants?



How do we know RhD variants?

Serology ⟷ **Genotype**



RhD polypeptide and RhD amino acid sequence from RESPIRE database

Real life scenarios…

Kim
16 yr old

Pre-op scoliosis

- Group A
- D testing: 1+
- 2nd D testing: 3+ (2nd antiserum)

⇩

Different strengths of reactions

Anna
28 yr old

G2P1A0
11 weeks pregnant

G1 (2018): O+ (ON)
G2 (2022): O-
1st analysis (QC)

⇩

Discrepant Rh group

Mary
64 yr old

G0P0A0
AML de novo

2016: AB+, screen -
2022: AB+, anti-D

⇩

How can she have an anti-D if she is D+?

Work Group on *RHD* genotyping (AABB-CAP)

COMMENTARY

It's time to phase in *RHD* genotyping for patients with a serologic weak D phenotype

S. Gerald Sandler,[1] Willy A. Flegel,[2] Connie M. Westhoff,[3] Gregory A. Denomme,[4] Meghan Delaney,[5] Margaret A. Keller,[6] Susan T. Johnson,[7] Louis Katz,[8] John T. Queenan,[9] Ralph R. Vassallo,[10] and Clayton D. Simon[11]

Transfusion 2015; 55:680-689

Recommendations

- 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- 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

2015 recommendations

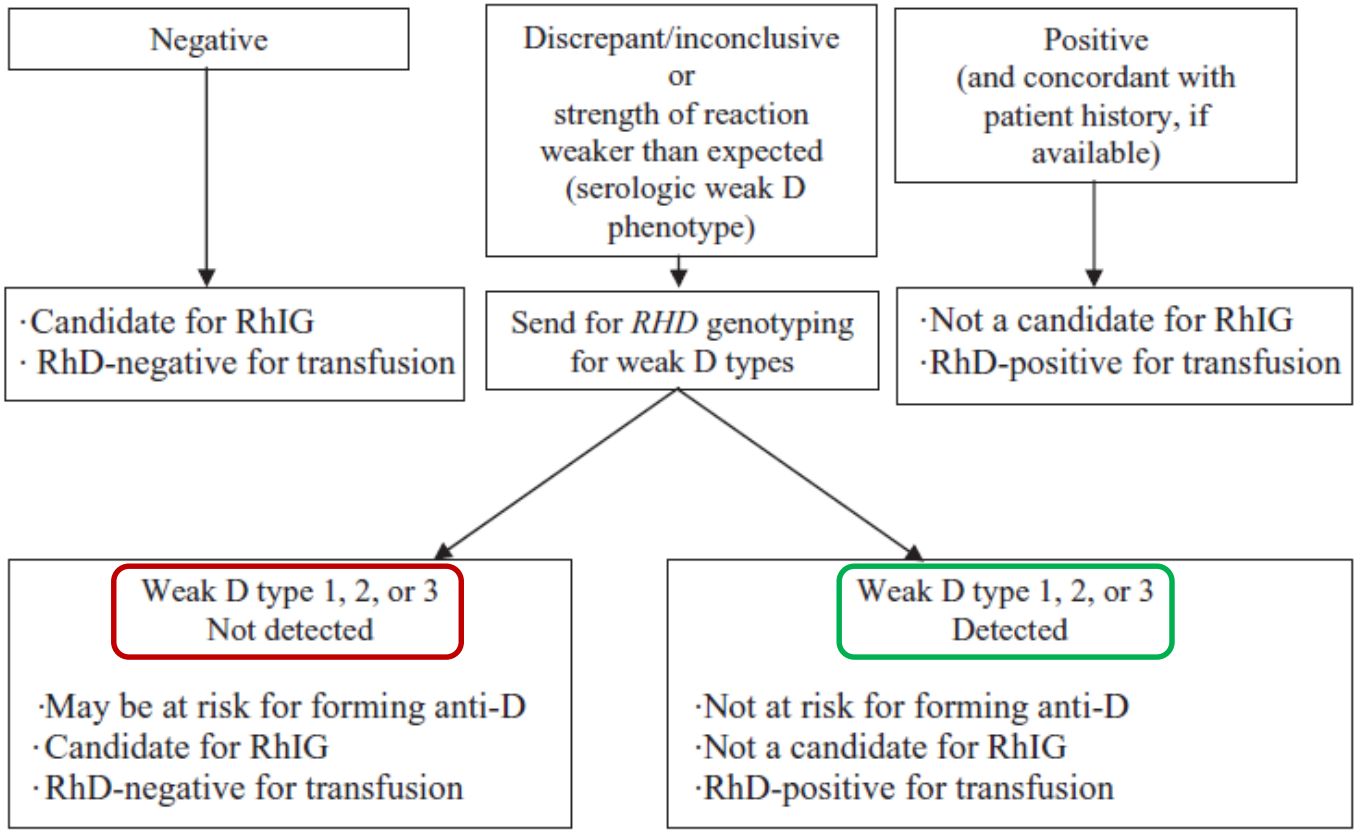
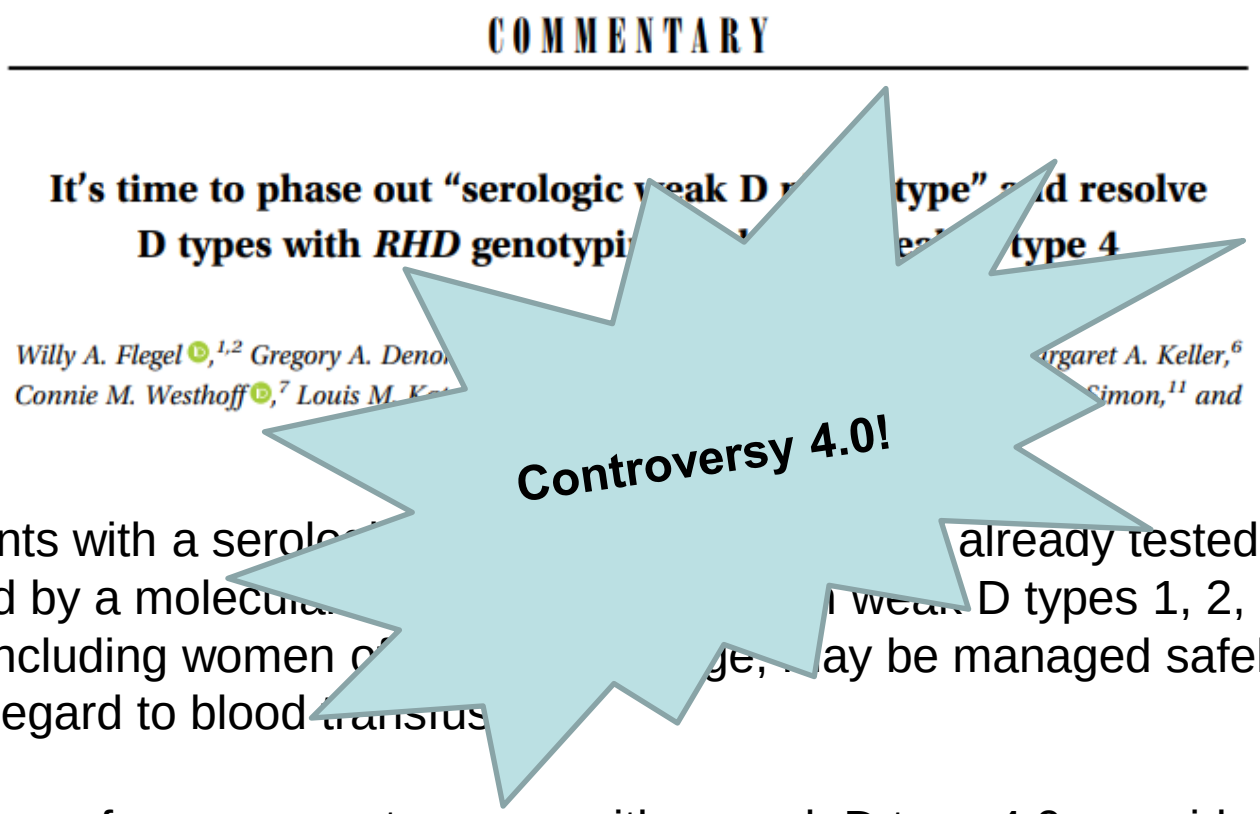


Fig. 3. Algorithm for resolving serologic weak D phenotype test results by *RHD* genotyping to determine candidacy for RhIG and RhD type for transfusions.

Transfusion 2015; 55:680-689

Updated recommendations: 2020


COMMENTARY

It's time to phase out "serologic weak D ... type" and resolve D types with *RHD* genotyping ... type 4

Willy A. Flegel, Gregory A. Deno... Margaret A. Keller, Connie M. Westhoff, Louis M. K... Simon, and

Controversy 4.0!

- Patients with a serolo... already tested, should be tested by a molecula... weak D types 1, 2, 3, **4.0** and **4.1**, including women o... ge, may be managed safely as D+ with regard to blood transfus...

- However, for a pregnant woman with a weak D type 4.0, consideration may be given for D− transfusions and RhIG for D immunoprophylaxis in an abundance of caution.

Transfusion 2020;60;855–859

Canadian recommendations

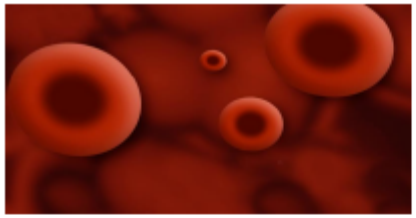
National Advisory Committee on Blood and Blood Products | Comité consultatif national sur le sang et les produits sanguins

Blood Shortage | Endorsements | Guidelines & Recommendations | Links & Resources

Home / Guidelines & Recomme... / Special Testing / Mo... / RHD Genotyping in Prenatal Patients

Guidelines & Recommendations

RHD Genotyping in Prenatal Patients

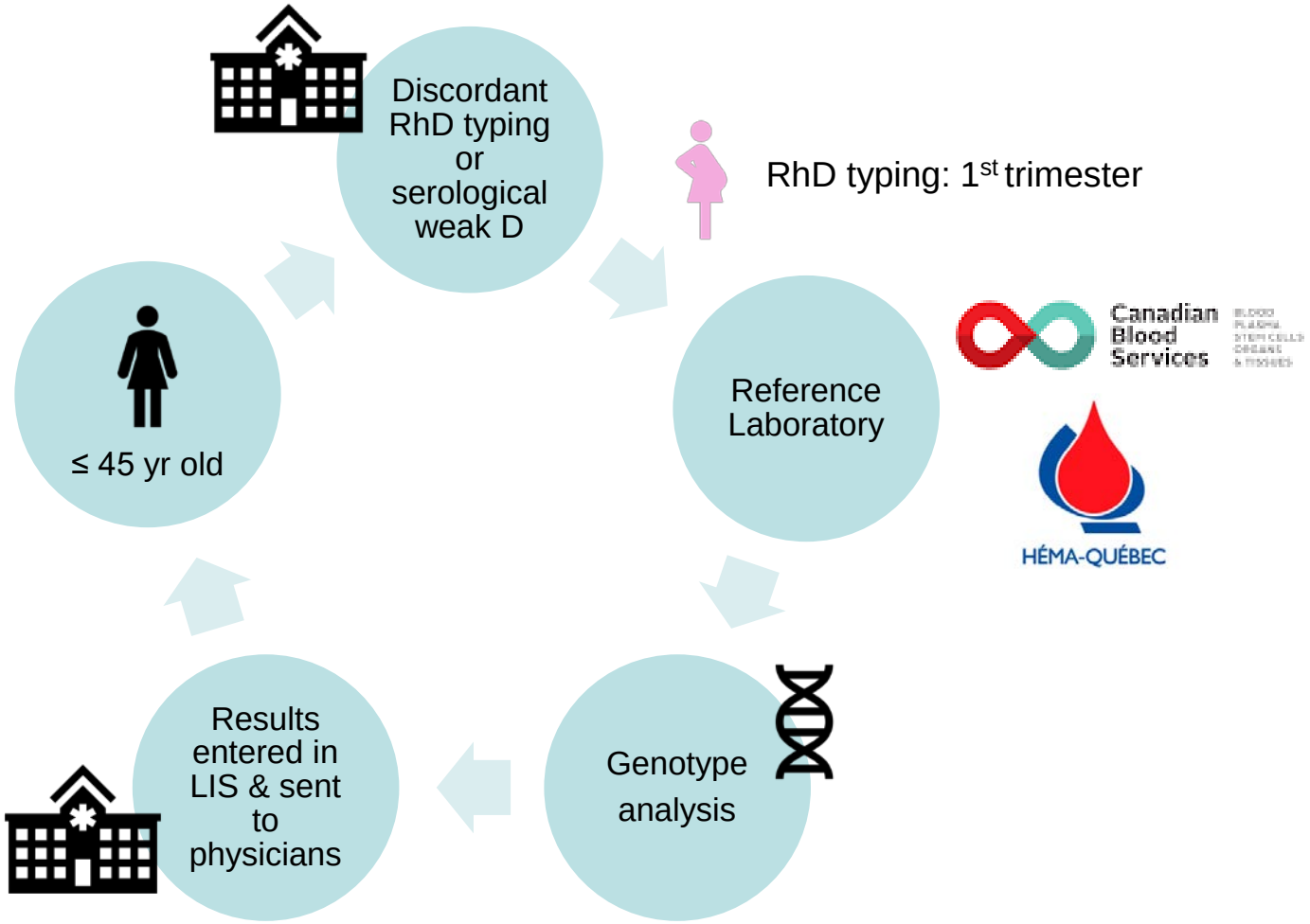


Recommandations pour la détermination du groupe sanguin RhD

Direction de la biovigilance et de la biologie médicale
Février 2016
Révision juin 2020

Québec

Genotype testing: the « how to »



Genotype testing: practical aspects

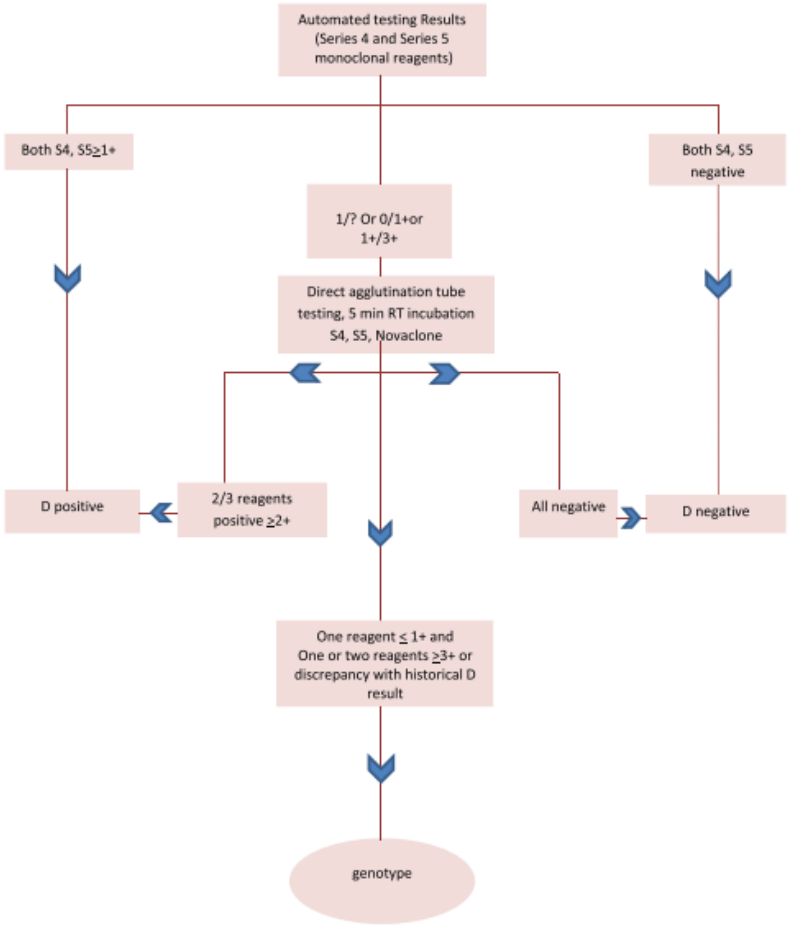
	Canadian Blood Services	Héma-Québec
Genotype testing Platform used	Immucor RHD Molecular BeadChip Test	RFLP & SSP Weak D type 1,2,3 and 42 (moving toward Immucor)
Samples required	One 2-7 ml EDTA tube	One 2-7 ml EDTA tube
Shipping requirement	T° ≥1°C Arrive to testing site < 48 hrs	Ice-pack
Results turn around time	Within 2 weeks	Within 2 weeks
Accessibility of results	Results available to requesting hospital	Shared LIS – results available to all QC hospitals
Testing site	Edmonton	Montreal

https://www.blood.ca/fr/node/7955

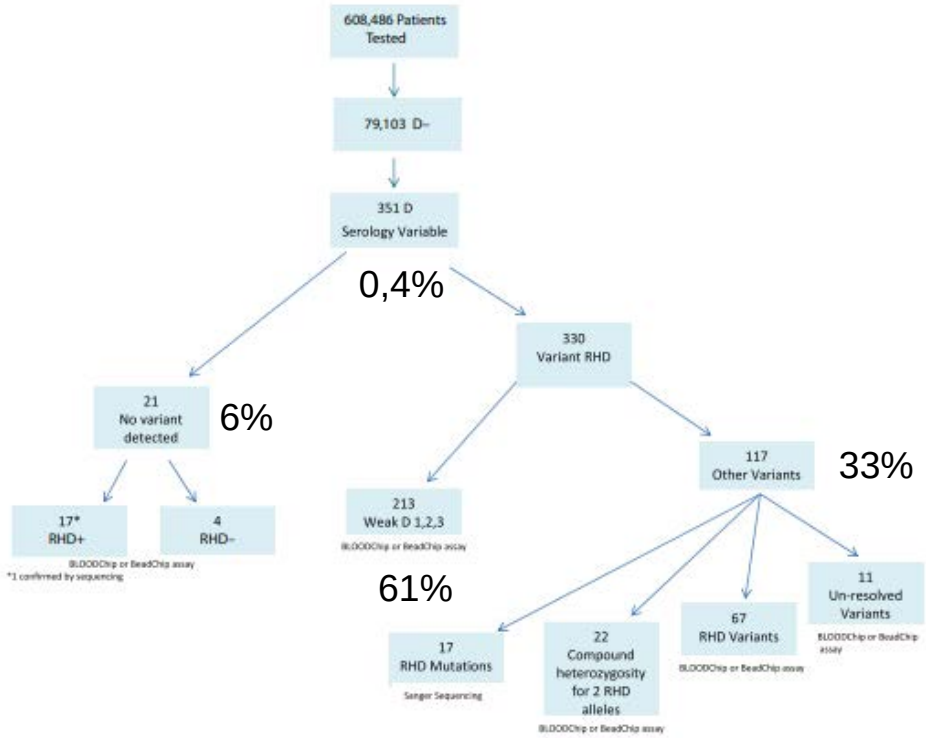
https://www.hema-quebec.qc.ca/userfiles/file/media/francais/cellulessouches/ENR-02681.pdf

Genotyping in prenatal patients: CBS experience

Prenatal D typing algorithm



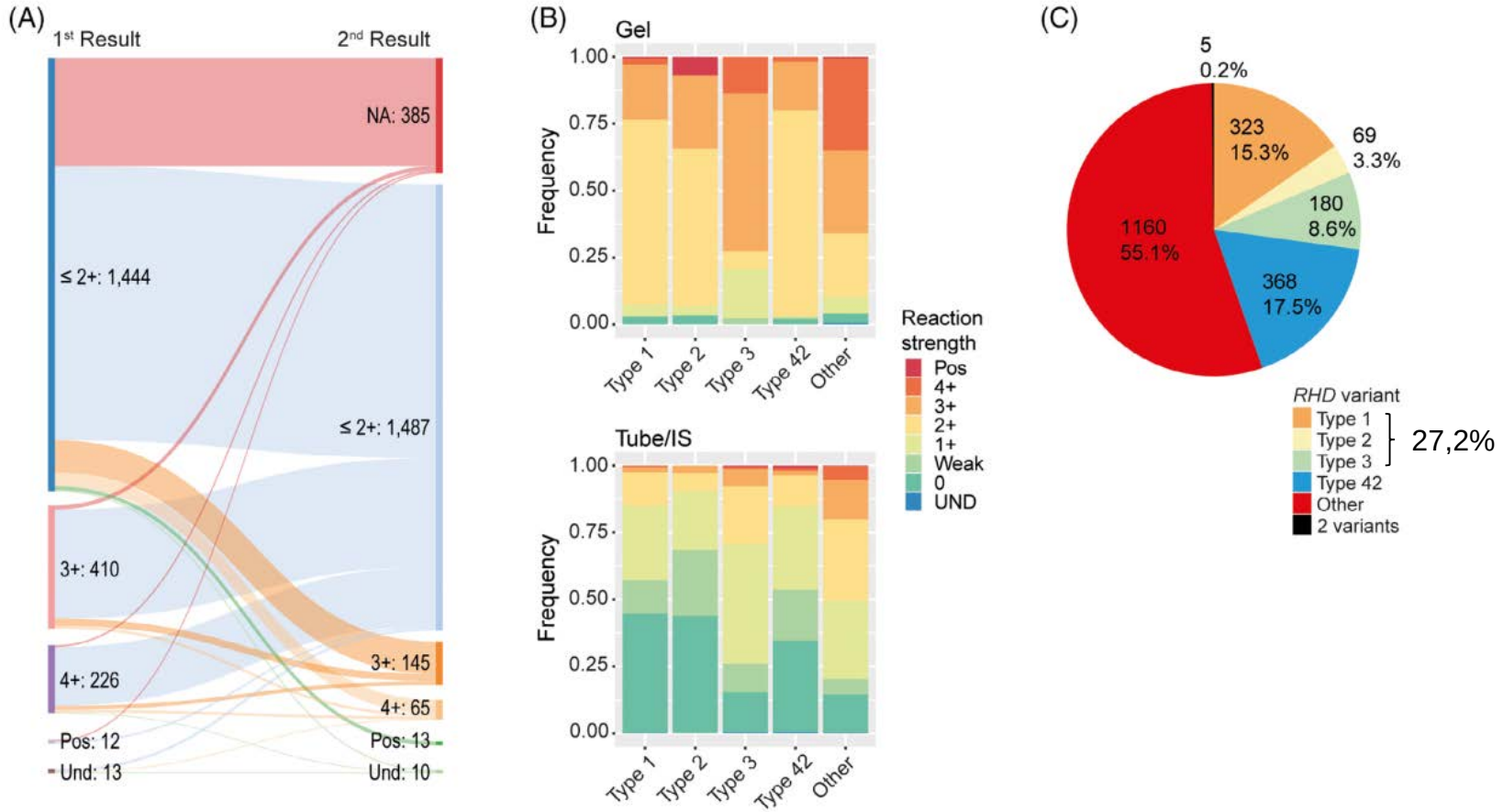
RHD genotyping results among D–prenatal patients with weak or variable D serology



Clark G et al, Transfusion 2016;56;2980–2985

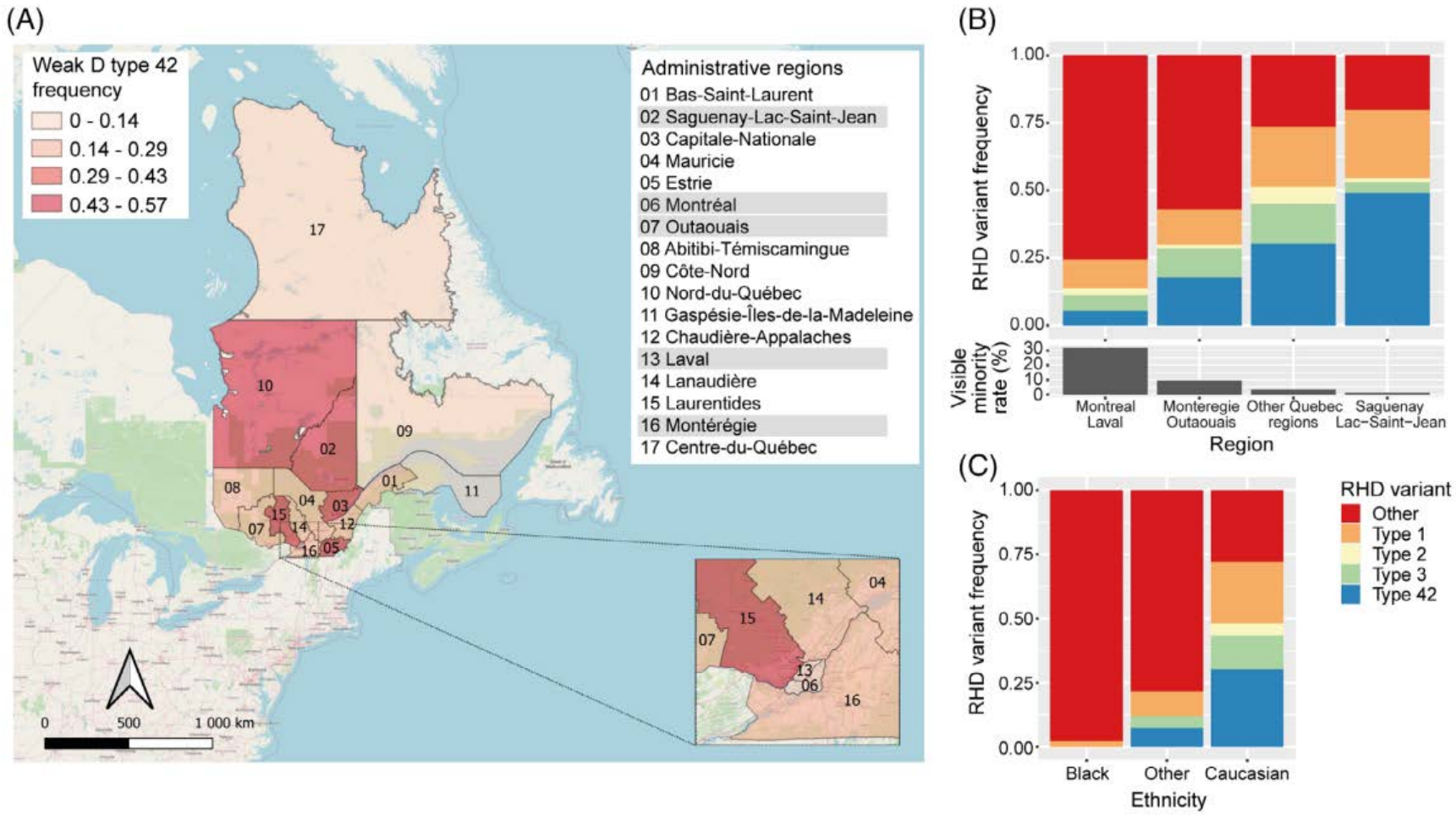
Genotyping in women ≤ 45 years old: Héma-Québec experience

Serological and genetic profile of weak D referred to Hema-Quebec's IRL



Leiva-Torres GA et al, Transfusion. 2021;61:2727–2735

Distribution of the RHD*01W.42 allele in the Quebec population



Leiva-Torres GA et al, Transfusion. 2021;61:2727–2735

While waiting for the results…

- What should I do with respect to RhIg prophylaxis for bleeds that occur in patients with weakly reactive RhD and genotype results are not available?
  1. Contact your IRL, the result may be available…
  2. If not, use a precautionary approach and give RhIg at appropriate dose
     - Case reports of HDFN caused by partial D
  3. No clear data in the literature to evaluate whether RhIg can prevent alloimmunization in patients with partial D

Quantock KM et al, Transfusion. 2017;57(8):1938-1943
Lukacevic KJ et al, Transfus Med Hemother. 2016;43(6):419-424
Turley E et al, Transfusion. 2018;58(10):2260-2264

Real life scenario: Kim

Kim
16 yr old

Pre-op scoliosis

- Group A
- D testing: 1+
- 2nd D testing: 3+ (2nd antiserum)

⇩

Different strengths of reactions

IRL

Genotyping results:

Weak D type 1

Clinical implications

Should be considered as D+

Transfuse with **D+** RBC

Not a Candidate for RhIg

Real life scenario: Anna

Anna
28 yr old

G2P1A0
11 weeks pregnant

G1 (2018): O+ (ON)
G2 (2022): O- (QC)

Ask for a 2nd sample and used a 2nd antiserum: w/-

⇩

Discrepant Rh group

IRL

Genotyping results:

Partial D type VI

Back in time…

2018: O- in IS but
O+ doing a weak D test

Clinical implications

Should be considered as D-

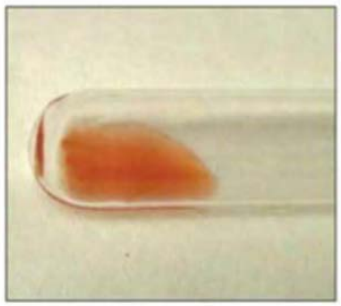
Transfuse with **D-** RBC

Candidate for RhIg

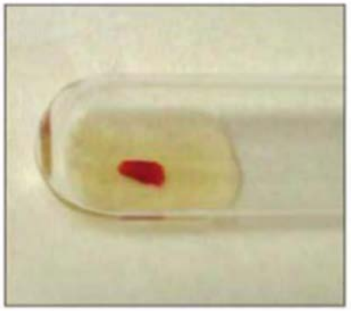
D testing

- Most reagents are « blended » mixture
  - Contain both IgG and IgM
    - Monoclonal IgM
    - Monoclonal or polyclonal IgG
  - Results read after immediate spin (IS)

Reagent anti-D + patient RBCs ⇨ centrifuge and examine for agglutination

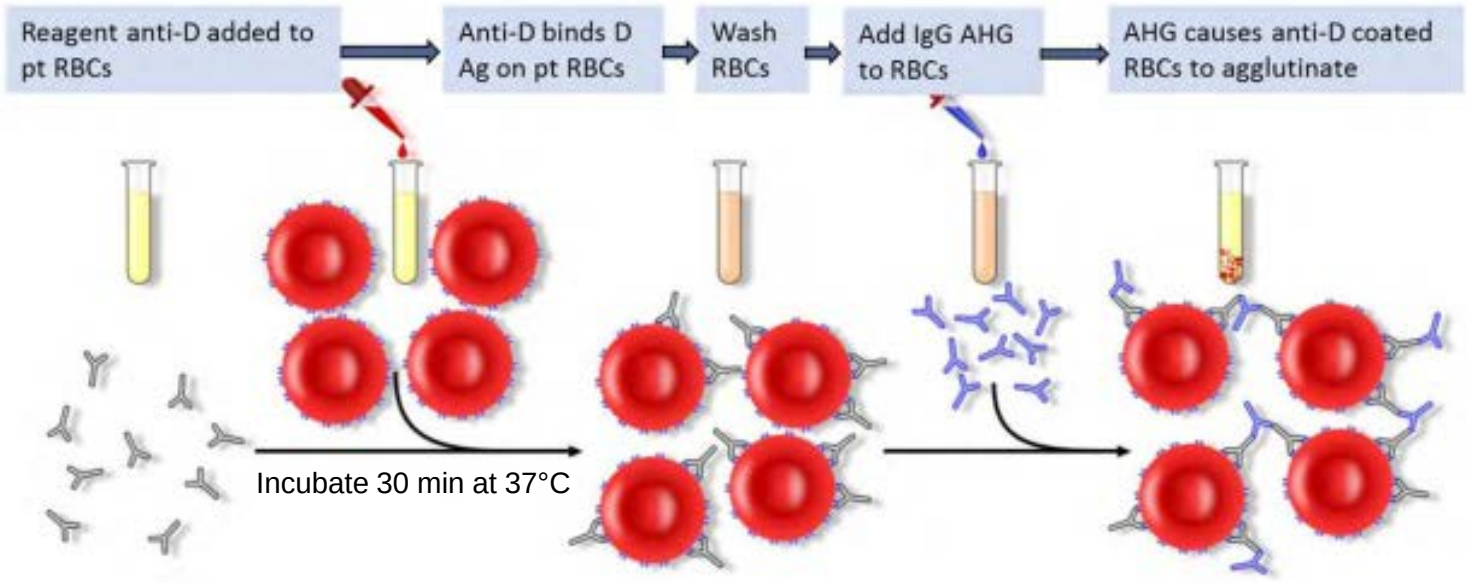


Negative reaction



4+ Reaction

Weak D test



Indications:

- D- blood donors
- Infants born to Rh D negative mothers (RhIg prophylaxis)

Image adapted from: Zarandona JM and Yazer MH. The role of the Coombs test in the evaluation of hemolysis in adults. Canadian Medical Association Journal 2006;174:305-307

Real life scenario: Mary

Mary
64 yr old

G0P01A0
AML

2016: AB+, screen -
2022: AB+, anti-D

⇩

How can she have an anti-D if she is D+?

IRL

Genotyping results:

Not indicated per the recommendations

But, academically…

Partial D type DVa

Clinical implications

Should be considered as D-

Transfuse with **D-** RBC as she has an anti-D

Conclusions

- Persons 45 years old and under, with childbearing potential, should be genotyped whenever inconclusive/discordant *RHD* typing results and/or serologic weak D phenotype is detected.
  - Samples should be sent to IRL with genotyping expertise.

- Persons with weak D type 1, 2, 3 and 4.1 should be considered as RhD-positive for transfusion and are not candidates for RhIg.
  - Weak D type 4.0 is still controversial.

Recommendation

STATEMENT 3

Weak or variably reactive D reactions in pregnant patients should be investigated with RHD genotyping.

Serologic weak D testing should not be performed.

Acknowledgments

- Melanie Bodnar, CBS
- Gabriel André Leiva-Torres, HQ

Questions?

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