Prevention & management of ALLOIMMUNIZATION in pregnancy
The PRAMS Program

Dr. Sarah Tehseen MBBS, Msc. FRCPC
Pediatric Hematology and Transfusion Medicine
Dr. Eman Ramadan MD. FRCPC
Maternal Fetal Medicine

Land acknowledgement

- *I acknowledge that we are gathering on Treaty 2, 4, 5, 6, 8 and 10 territories and the homeland of the Metis.*
- *Recognizing this history is important to our future and our efforts to close the gap in health outcomes between Indigenous and non-Indigenous peoples. I pay my respect to the traditional caretakers of this land.*

Objectives

- What is the definition of alloimmunization? Why does it matter?
- Who is at risk for alloimmunization? How do we identify them?
- What is RhIg? When is RhIg indicated during pregnancy?
- What is the PRAMS program?

Basics of Alloimmunization in pregnancy

Question

You receive lab results for one of your pregnant patients from the first prenatal visit at 12 weeks gestation.

What are your next steps?

ABO	O
Rh	Positive
Antibody Screen	Positive

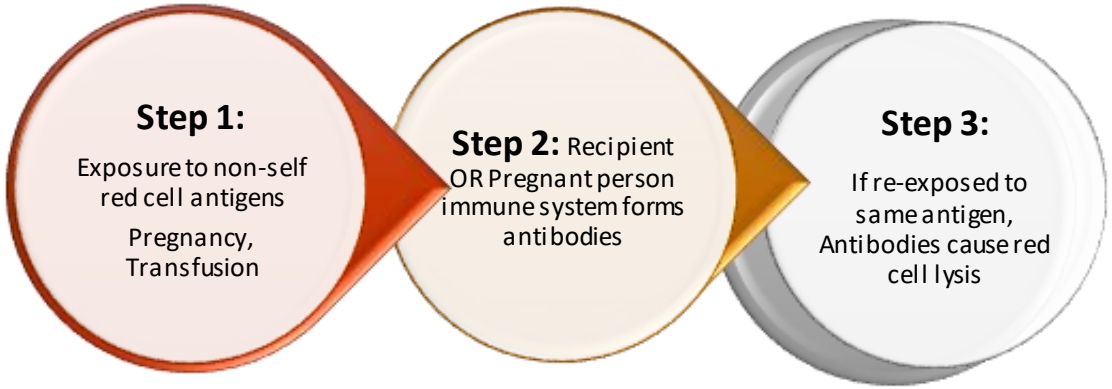
Blood Group antibody Identification	POS Anti-E Cnote

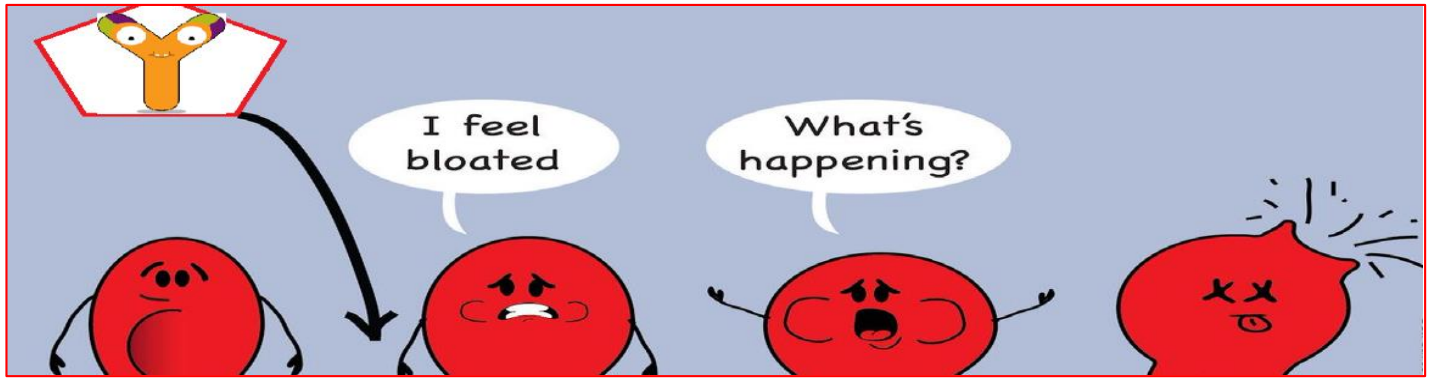
Anti-E (big) (titer 8) has been implicated in HDFN. Critical titer has not been reached. Submit follow-up sample in 4 weeks."

Father's sample is required for ABO-Rh and red cell phenotyping. Include expectant patient's name and HSN on the requisition when submitting the father's sample."

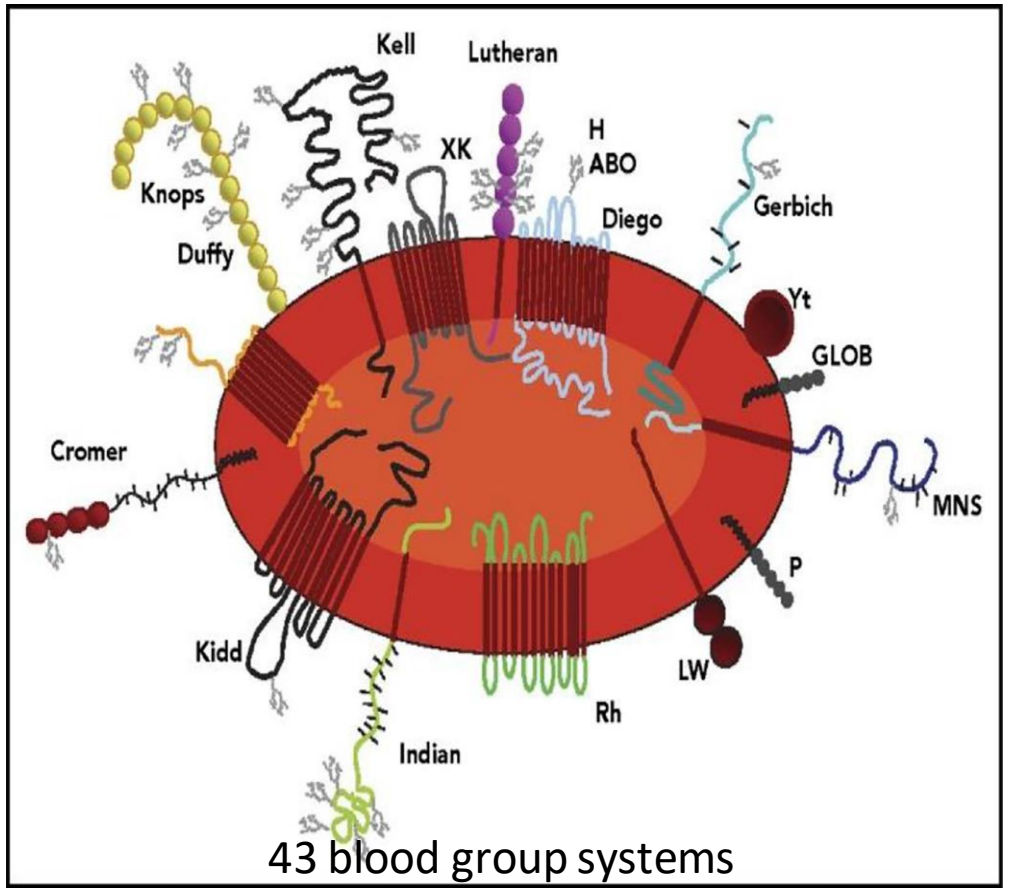
Back to the Basics-Alloimmunization

Development of antibodies to cell surface proteins (antigens) from a different member of the same species.





So how many red cell antigens are there?



43 blood group systems

All Red cell antigens and antibodies are not created equal

Occurrence and severity of HDFN depends on

Can the antibody cross placenta? IgG vs. IgM

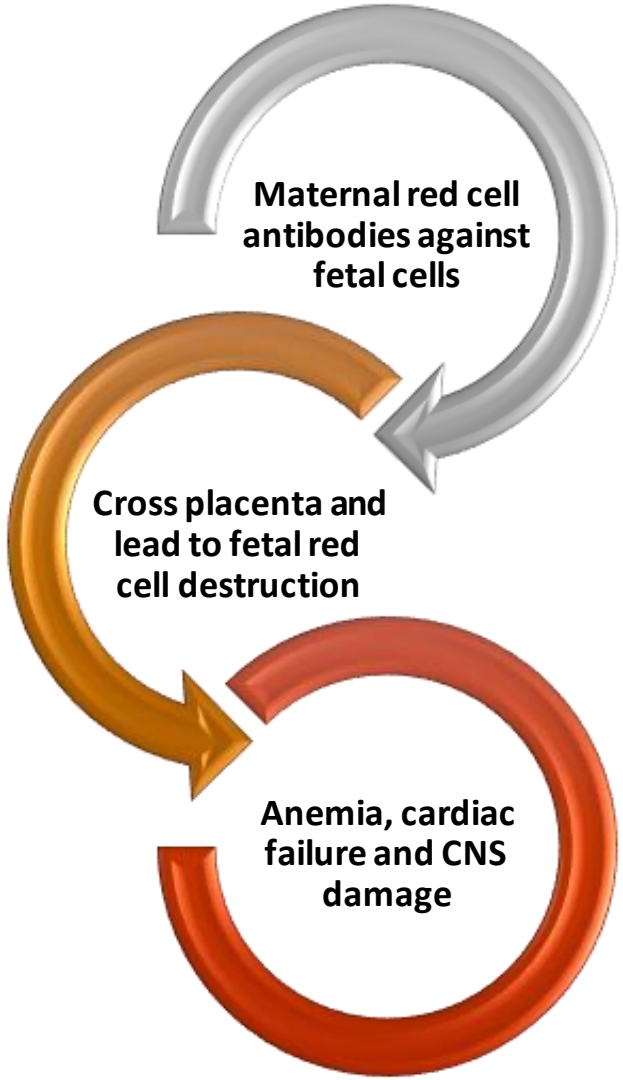
When is the red cell antigen expressed on fetal RBCs?

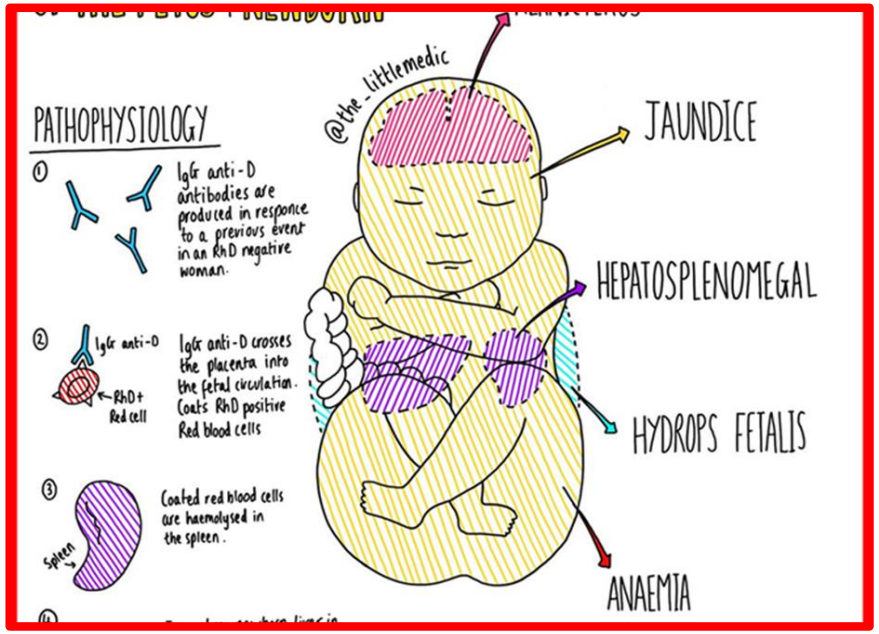
No. of antigen sites on fetal RBCs'.

Protein size/shape

Smart, Elizabeth, Beryl Armstrong, and Reviewer for Second Edition: Edmond Lee. "Blood group systems." *ISBT Science Series* 15 (2020): 123-150.

What does it look like in pregnancy





Vast Majority of still births or fetal anemia requiring intra-uterine transfusions occur due to

Anti-D

Anti-c (little)

Anti-Kell

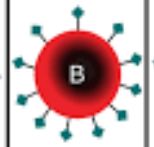
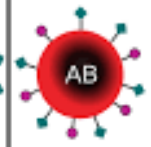
Surveillance of alloimmunization in pregnancy

Who are the prenatal patients at risk of alloimmunization?

- We have NO idea
  - Well we have SOME idea
- What we know about people who form anti-red cell antibodies
  - More likely to have autoimmune conditions
  - Have already formed one antibody

All pregnant individuals should undergo transfusion testing at initial clinic visit (usually at 10-15 weeks GA) which includes

Maternal ABO, Rh typing

	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies in plasma	 Anti-B	 Anti-A	None	 Anti-A and Anti-B
Antigens in red blood cell	A antigen	B antigen	 A and B antigens	None

Antibody screen

	Rh	C	c	D	E	e	C^w	M	N	S	s	P1	K	Lea	Leb	Fya	Fyb	Jka	Jkb	IAT
1	R_1R_1	+	-	+	-	+	-	-	+	-	+	+	-	+	-	+	-	-	+	-
2	R_2R_2	-	+	+	+	-	-	-	+	-	+	-	+	-	+	+	-	+	-	+
3	rr	-	+	-	-	+	-	+	-	+	-	+	-	-	+	-	+	+	-	+

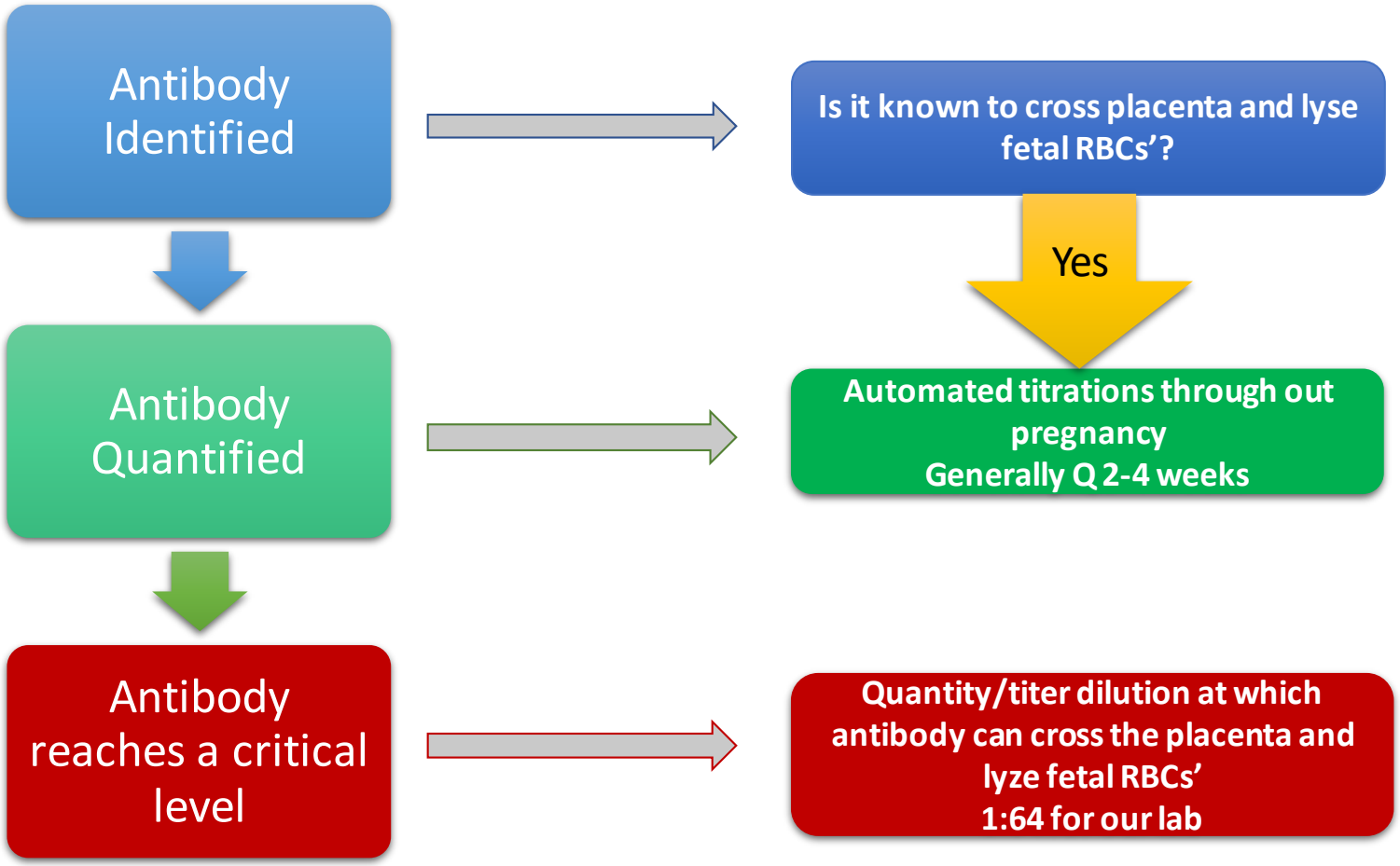
Antibody identification panel

	Rh	C	c	D	E	e	C^w	M	N	S	s	P1	K	Lea	Leb	Fya	Fyb	Jka	Jkb	IAT	Enz
1	$R_1^wR_1$	+	-	+	-	+	+	+	-	+	-	+	-	+	-	-	+	-	+	-	-
2	R_1R_1	+	-	+	-	+	-	+	+	+	+	-	+	-	+	+	-	+	-	-	-
3	R_2R_2	-	+	+	+	-	-	+	-	+	-	+	-	+	-	+	-	+	-	+	+
4	r'r	+	+	-	-	+	-	-	+	-	+	+	-	-	+	-	+	-	+	+	+
5	r''r	-	+	-	+	+	-	+	+	+	+	+	-	-	+	+	-	+	-	+	+
6	rr	-	+	-	-	+	-	-	+	-	+	-	-	-	+	-	+	-	+	+	+
7	rr	-	+	-	-	+	-	-	+	-	+	+	+	+	-	-	+	+	-	+	+
8	rr	-	+	-	-	+	-	+	-	+	-	+	-	-	+	+	-	-	+	+	+
9	rr	-	+	-	-	+	-	+	-	-	+	-	-	-	-	+	-	-	+	+	+
10	rr	-	+	-	-	+	-	-	+	+	-	+	+	-	+	+	-	-	+	+	+

Red cell antibody screen

Antibodies against minor red cell antigens in maternal plasma

General Monitoring process for alloimmunization



The same patient is now 28 weeks gestation. You have diligently ordered transfusion testing every 4 weeks and even asked the dad to get tested. Following comment comes today

Anti-E (big) (titer 128) has been implicated in HDFN. Critical titer HAS been reached. Urgent referral to Maternal Fetal Medicine Specialist is strongly recommended. No further specimens required in this pregnancy.

Father: Last, First Name (HSN XXX XXX XXX) has been tested as homozygous positive for E (big) antigen on DD/MON/YYYY. If paternity is assured, baby's red cells WILL be positive for E antigen and HDFN due to anti-E is a concern in this pregnancy.

Critical Antibody titer

Antibody level/quantity in maternal plasma is high enough to cross placenta and cause fetal hemolysis

Different testing methodologies have different cut-offs. 1:64 for gel.

Does it mean fetal hemolysis is occurring?

No! It means, hemolysis **CAN** occur.

Your friendly MFM ascertains presence of fetal anemia through fetal MCA Doppler

Hence Critical Titer = <u>Referral to MFM</u>

Some red cell antibodies just like to be different

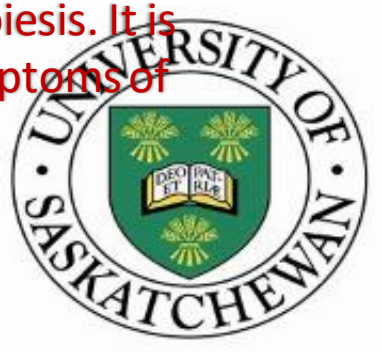
- Prenatal booking antibody screen for a G2P1 female is reported as follows at 20 weeks GA:

ABO	**O**
Rh	Positive
Antibody Screen	Positive
Blood group AB identification	Anti-Kell Cnote

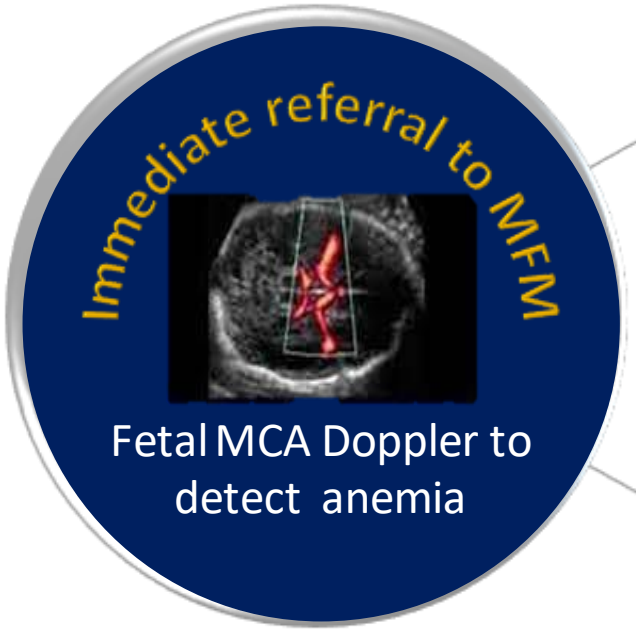
Anti-Kell has been implicated in HDFN. Detection of anti-Kell is a critical result regardless of titer strength. Referral to Maternal Fetal Medicine Specialist is strongly recommended. Submit follow-up sample at 26-28 weeks."

Father's sample is required for ABO-Rh and red cell phenotyping. Include expectant patient's name and HSN on the requisition when submitting the father's sample."

"Anti-Kell may cause suppression of fetal erythropoiesis. It is recommended that the baby be monitored for symptoms of late-onset anemia up to 2 months of age ."



Certain scenarios warrant MFM referral before antibody reaches critical titer



Anti-Kell antibody Detection

- Crosses placenta at very low titers
- Exact threshold not established

Positive antibody ID in a patient with prior HDFN due to same AB

- Intra-uterine transfusion, neonatal exchange or straight transfusion in the past
- Antibody titers unreliable for determining risk of fetal transfer and hemolysis

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The variability of ‘D’ antigen

Weak in the Ds'

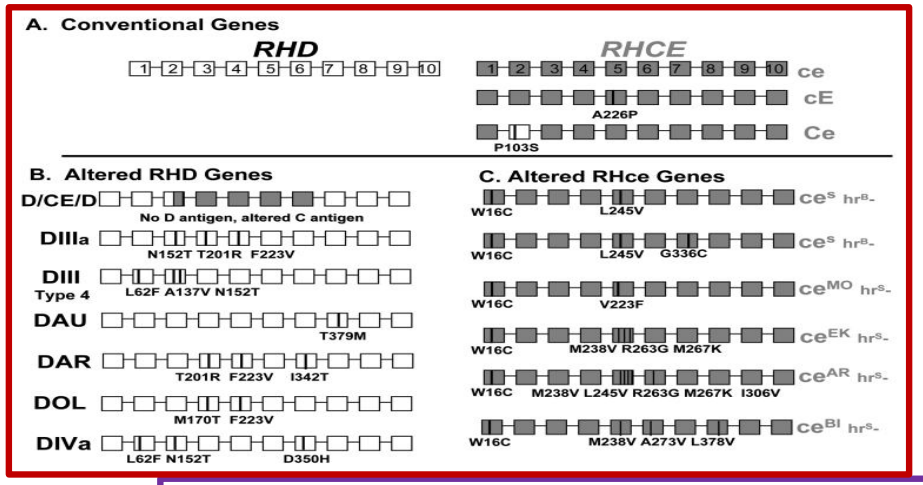
You are following a G2P1 pregnant patient. She just underwent her initial prenatal transfusion testing. You got the following results:

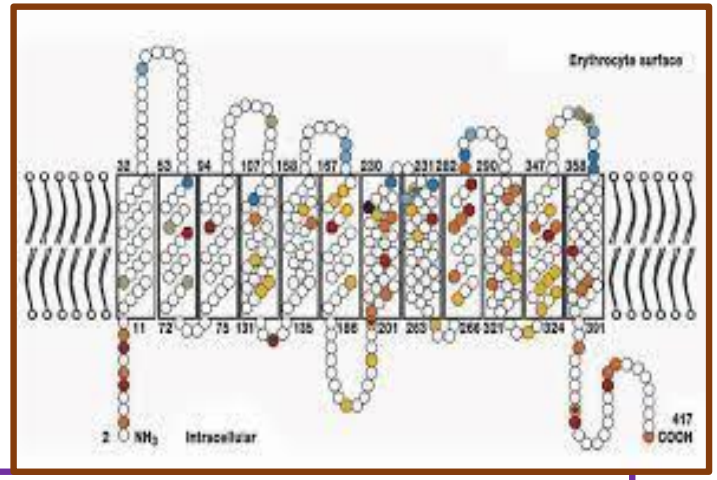
ABO	A
Rh	Indet. (short for indeterminate)
Antibody Screen	Negative

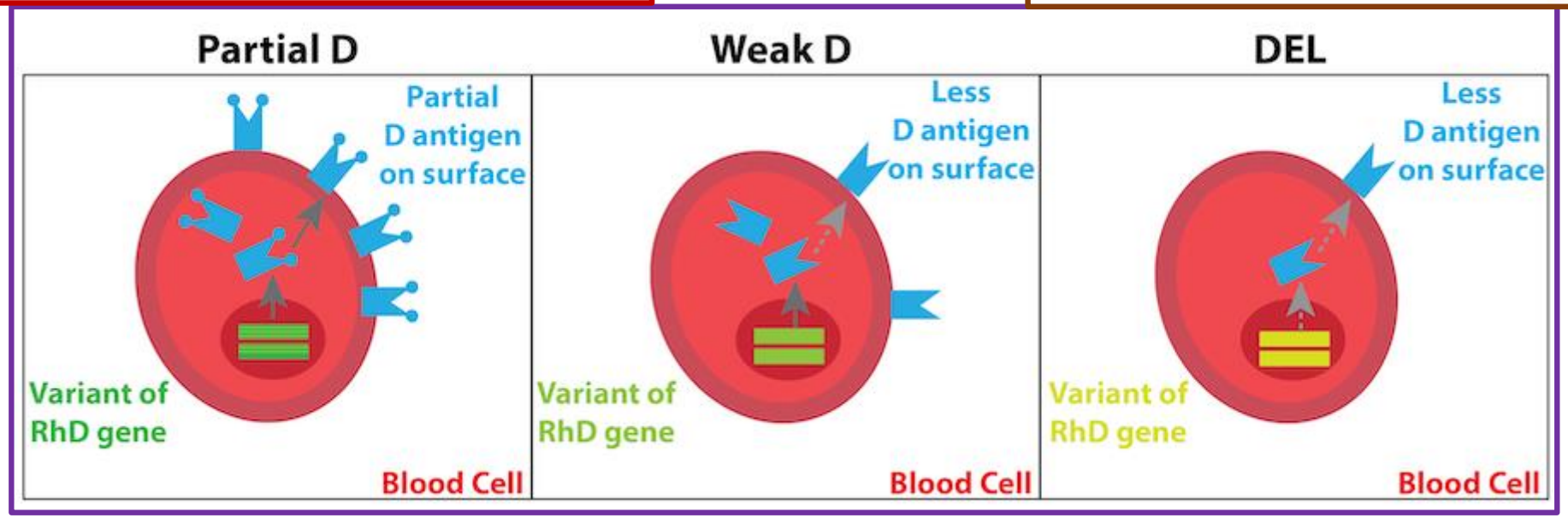
Rh Indeterminate. Sample sent for genotyping. Until Rh type is determined, consider patient as Rh negative and eligible for RhIg. Recommend follow-up sample two weeks prior to 28 week RhIg administration.

The patient tells you 'Ummmm I thought I was Rh positive'

Rh (D) Variants-Just for understanding







https://www.thetech.org/ask-a-geneticist/rh-blood-test-accuracy
Westhoff CM. The structure and function of the Rh antigen complex. Seminars in Hematology. 2007 Jan;44(1):42-50. DOI: 10.1053/j.seminhematol.2006.09.010. PMID: 17198846; PMCID: PMC1831834.

How do we know which is which?

RHD Genotyping at CBS
This information is constantly updated

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The comment from CBS comes back 6 weeks later

Test Performed:	Results:	Comment:
RHD Genotyping	Weak D type 4.0 or 4.3 (RHD*09.03 or RHD*09.05)	

Remarks:
RHD genotyping results indicate that this patient is unlikely to form alloanti-D. However, per current practice recommendations, the patient should be treated as an RhD negative individual. Testing was performed using Immucor's RHD Molecular BeadChip Test which is an in vitro diagnostic test licensed by Health Canada.

It can ALSO be resulted as the comment below depending on Rh D variant

RHD Genotyping	Weak D type 1 (RHD*01W.1)

Remarks:
RHD genotyping results indicate that this patient will not form alloanti-D and should be treated as an RhD positive individual. Testing was performed using Immucor's RHD Molecular BeadChip Test which is an in vitro diagnostic test licensed by Health Canada.

Or like this

RHD Genotyping	DAR (RHD*09.01) / Weak D type 4.0 or 4.3 (RHD*09.03 or RHD*09.05)

Remarks:
RHD genotyping results indicate that this patient may form alloanti-D and should be treated as an RhD negative individual. Testing was performed using Immucor's RHD Molecular BeadChip Test which is an in vitro diagnostic test licensed by Health Canada.

RhIg Prophylaxis

The Pathophysiologic Basis

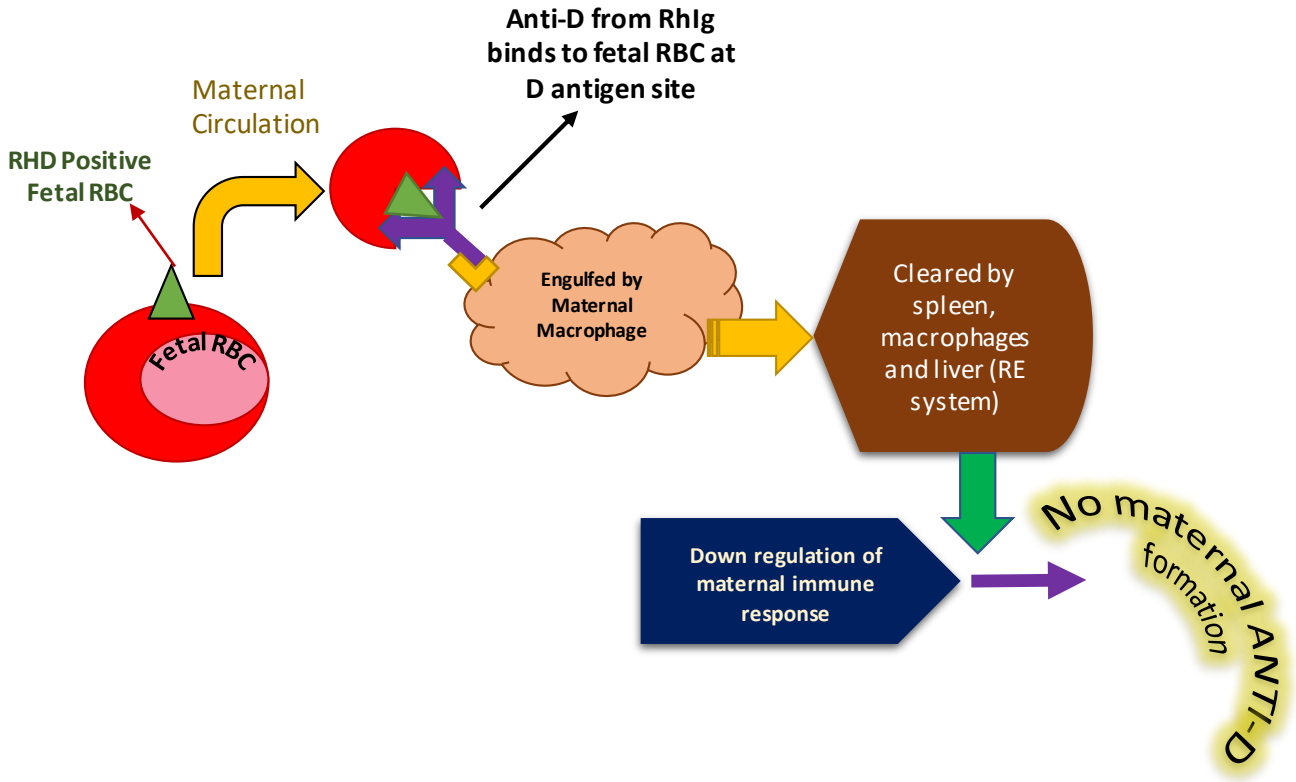
The conundrums of WinRho administration

A 25 year old G1 patient comes to your clinic for 20 week appointment. She is A negative. She tells you

‘I don’t want WinRho because its not natural and doesn’t work’

Role of WinRho in RhD alloimmunization

- Numerous mechanisms of action proposed-Not PROVEN



Plasma protein product derived from individuals with high titers of IgG antibodies to D-positive erythrocytes

Does it work?

- ❑ Alloimmunization rate in prenatal patients Pre-RhIG: 16%
- ❑ Post-Natal RhIg added: Rate dropped to 1-3%
- ❑ Prophylactic RhIg at 26-28 weeks GA: Rate of 0.2-0.3%

Bowman, J M. "The prevention of Rh immunization." Transfusion medicine reviews vol. 2,3 (1988): 129-50. doi:10.1016/s0887-7963(88)70039-5
Koelewijn, Joke M et al. "One single dose of 200 microg of antenatal RhIG halves the risk of anti-D immunization and hemolytic disease of the fetus and newborn in the next pregnancy." Transfusion vol. 48,8 (2008): 1721-9. doi:10.1111/j.1537-2995.2008.01742.x Huchet, J et al. "Application anté-partum du traitement préventif d'immunisation rhésus D chez les femmes rhésus négatif. Evaluation parallèle des passages transplacentaires d'hématies foetales. Résultats d'une étude multicentrique menée en région parisienne" [Ante-partum administration of preventive treatment of Rh-D immunization in rhesus-negative women. Parallel evaluation of transplacental passage of fetal blood cells. Results of a multicenter study carried out in the Paris region]. *Journal de gynecologie, obstetrique et biologie de la reproduction* vol. 16,1 (1987): 101-11.

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Also RhIg has no Mercury

RhIg dosing
Early Pregnancy

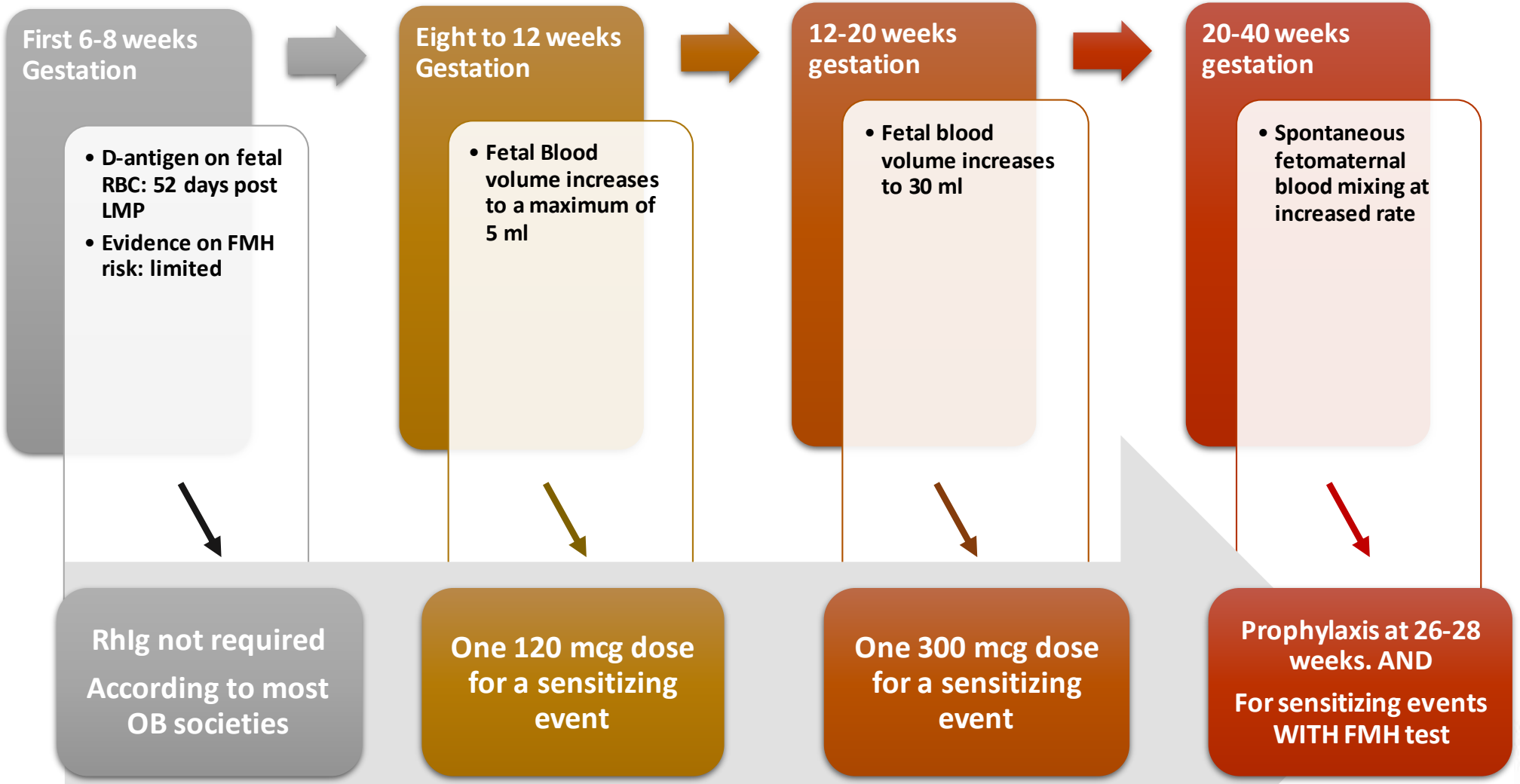
A 30 year old G1 Rh negative female presented to ER at 15 weeks gestation with PV bleeding.

Her type and screen showed that she is B negative with AB screen negative

Does she need RhIg?

- How much

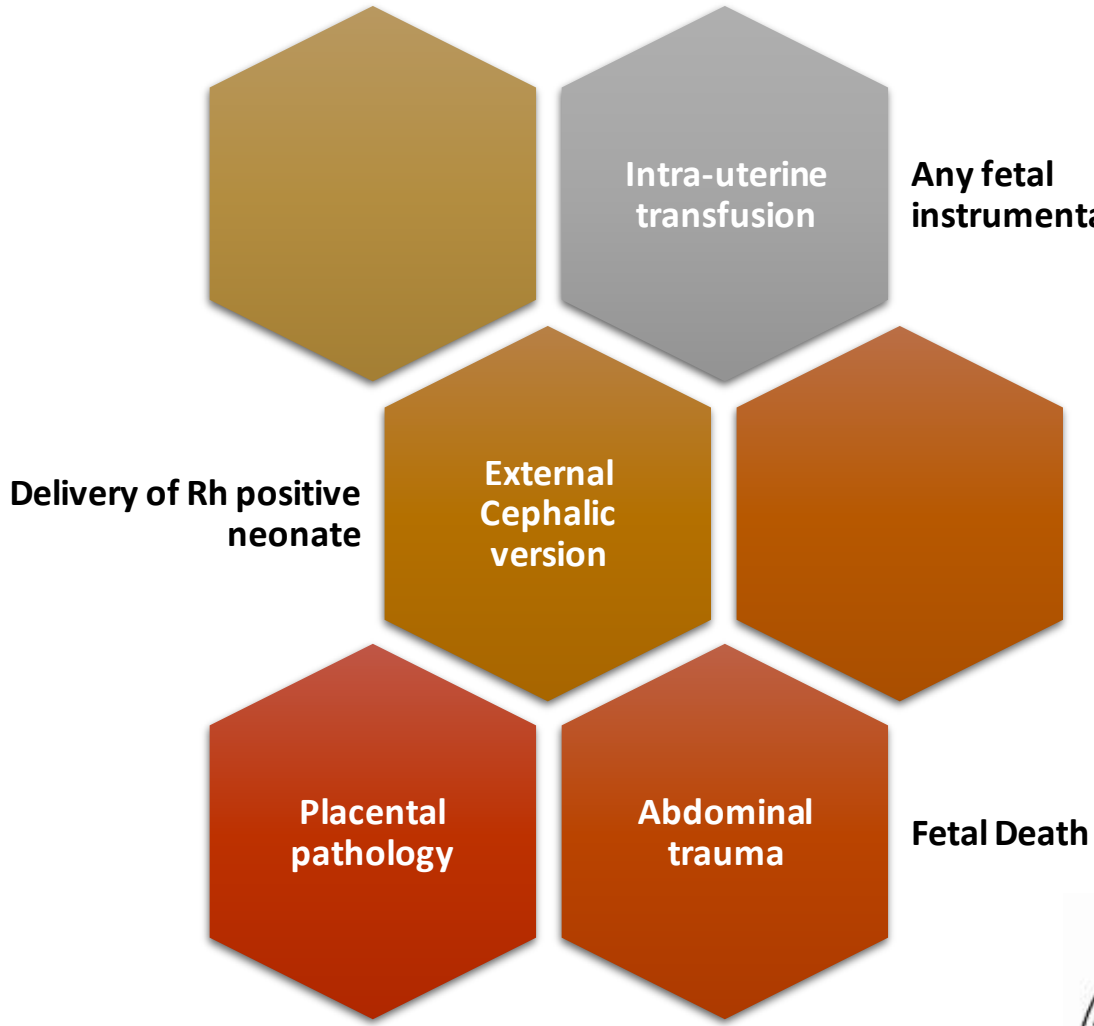
Administration based on gestational age



Judd, W. J., et al. "Prenatal and perinatal immunohematology: recommendations for serologic management of the fetus, newborn infant, and obstetric patient." Transfusion 30.2 (1990): 175-183.
Litwak, Oscar, et al. "Fetal erythrocytes in maternal circulation after spontaneous abortion." JAMA 214.3 (1970): 531-534.

What is a sensitizing event?

Event associated with a high risk of fetal maternal blood mixing and in large volumes



RhIg dosing
Late second and early 3rd Trimester

An Rh negative G1 woman presents to ER with vaginal bleeding due to placenta Previa. She is at 30 weeks gestation.

She received prophylactic RhIg 2 weeks ago. The ER orders a type and screen

ABO	**A**
Rh	Negative
Antibody screen	Positive

RhIg issued: (DD/MON/YYYY). Anti-D probably due to recent RhIg injection. Patient is eligible for RhIg. No further routine samples required in this pregnancy

The ER calls and asks ‘Does she need more WinRho?’

Prophylactic RhIg dose at 26-28 weeks protects against maternal D alloimmunization

Additional WinRho is needed for sensitizing events

Due to <u>PHYSIOLOGIC</u> mixing of fetal blood in maternal circulation which increases throughout pregnancy

Zipursky, Alvin, and Lyonel G. Israels. "The pathogenesis and prevention of Rh immunization." *Rhesus haemolytic disease*. Springer, Dordrecht, 1967. 253-266.

FMH and RhIg dose

Half Life of RhIg: 24 days.

<u>300 mcg of RhIg</u>

Protects against 15 ml of fetal RBCs' OR 30 ml of fetal whole blood

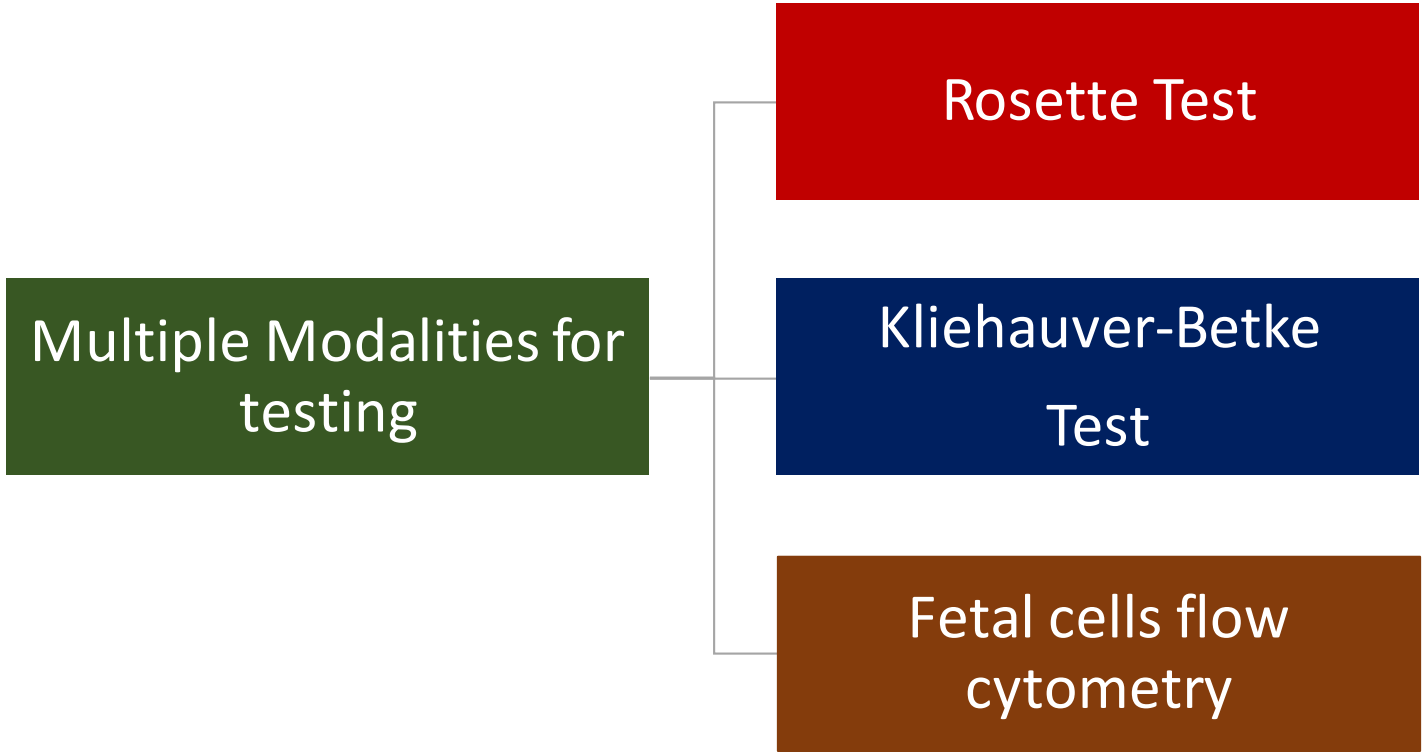
What if the fetal hemorrhage is larger than 30 ml

Passive anti-D due to WinRho is detected in the plasma 3-6 months post administration

Passive D ≠ Protection against D alloimmunization

Fung MK, Grossman BJ, Hillyer CD, Westhoff CM. Technical manual of the AABB. 18th ed. Bethesda, MD: AABB; 2014.
Sebring ES, Polesky HF. Fetomaternal hemorrhage: incidence, risk factors, time of occurrence, and clinical effects. *Transfusion* 1990;30(4):344-57.

Fetomaternal Hemorrhage Detection and quantification is done to determine additional RhIg dosing needing to cover large FMH instances



Fung MK, Grossman BJ, Hillyer CD, Westhoff CM. Technical manual of the AABB. 18th ed. Bethesda, MD: AABB; 2014.

More on FMH

Can it happen with ANY sensitizing event in a quantity large enough to need increase in RhIg dose?

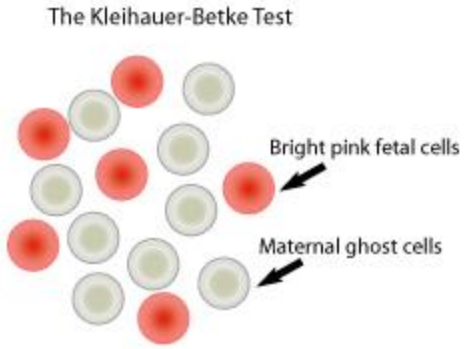
- Potentially
- Lack of concrete data

What are the situations where FMH is more likely to occur in large volume?

- **Highest risk in 3rd trimester**
- **Birth of Rh positive infant**
- Intra-uterine fetal death
- Placental Abruption
- **Pelvic trauma**
- **External Cephalic version**
- Twin gestation

Sebring ES, Polesky HF. Fetomaternal hemorrhage: incidence, risk factors, time of occurrence, and clinical effects. Transfusion 1990;30(4) Marcus R, Crewe-Brown H, Krawitz S, et al. Fetematernal hemorrhage following successful and unsuccessful attempts at external cephalic version. Br J Obstet Gynaecol 1975;82:578–80.
Nord E, Blaschke E, Green K, et al. 100 cases of external cephalic version with special reference to fetomaternal transfusion. Acta Obstet Gynecol Scand 1989;68:55–8.
Lau T, Stock A, Rogers M. Fetematernal hemorrhage after external cephalic version at term. Aust N Z J Obstet Gynaecol 1995;35: 173–4.
Pearlman M, Tintinalli J, Lorenz R. Blunt trauma during pregnancy. N Engl J Med 1990;323:1609–13.

Does your patient need more RhIg?



And How Much?

- 75 ml Number of fetal Cells counted: 30 fetal cells/2000 maternal cells
- Fetal RBCs %: 30/2000 = 1.5%
- Maternal Blood Volume: 5000 ml
- Total Fetal RBCs in maternal circulation: $\frac{1.5\ x\ 5000}{100}$ = 75 ml
- WinRho Dose is always Rounded up = $\frac{75}{30}$ = 2.6 = 4 Vials of RhIg

Calculation is done by TML technologists who then provide recommended RhIg dose

What if your patient FMH test showed no fetal cells?

- In ANY instance of sensitizing event, a standard dose of RhIg [300 mcg] must be given
- Requirement for additional dose depends on the FMH test results

Patient had received a Prophylactic RhIg dose 2 weeks ago.
Does it matter?

- NO!
- Prophylactic 26-28 week RhIg covers the ‘physiologic’ FMH for the remainder of pregnancy
- Any additional hemorrhage must be covered by additional RhIg

What if the patient comes with recurrent PV bleeding in 2nd/3rd trimester?

- Ambiguous recommendations
- RhIg has a ½ life of 24 days
- RhIg can be given every 3-6 weeks
  - A standard 300 mcg dose
    - With FMH testing
- Consider OB/MFM consult for best circumstantial advice

RhIg dosing-Delivery

A 30 year old G1 person delivers a neonate at 39 weeks GA.

She had received RhIg 6 weeks ago for bleeding due to placenta Previa. Cord blood results are provided below

ABO	**O**
Rh	Positive
DAT	Positive

Does the post-natal patient require RhIg?

Why is the DAT positive for neonate?

IS WinRho Indicated

- Yes. Half life of RhIg = 24 days. Highest risk of alloimmunization at delivery of Rh Positive infant (15-20%).
- A standard dose can be withheld: FMH test negative AND RhIg given ≤ 3 weeks ago.

Is FMH testing required

- Yes. Risk of large volume FMH at delivery is 2-3/1000

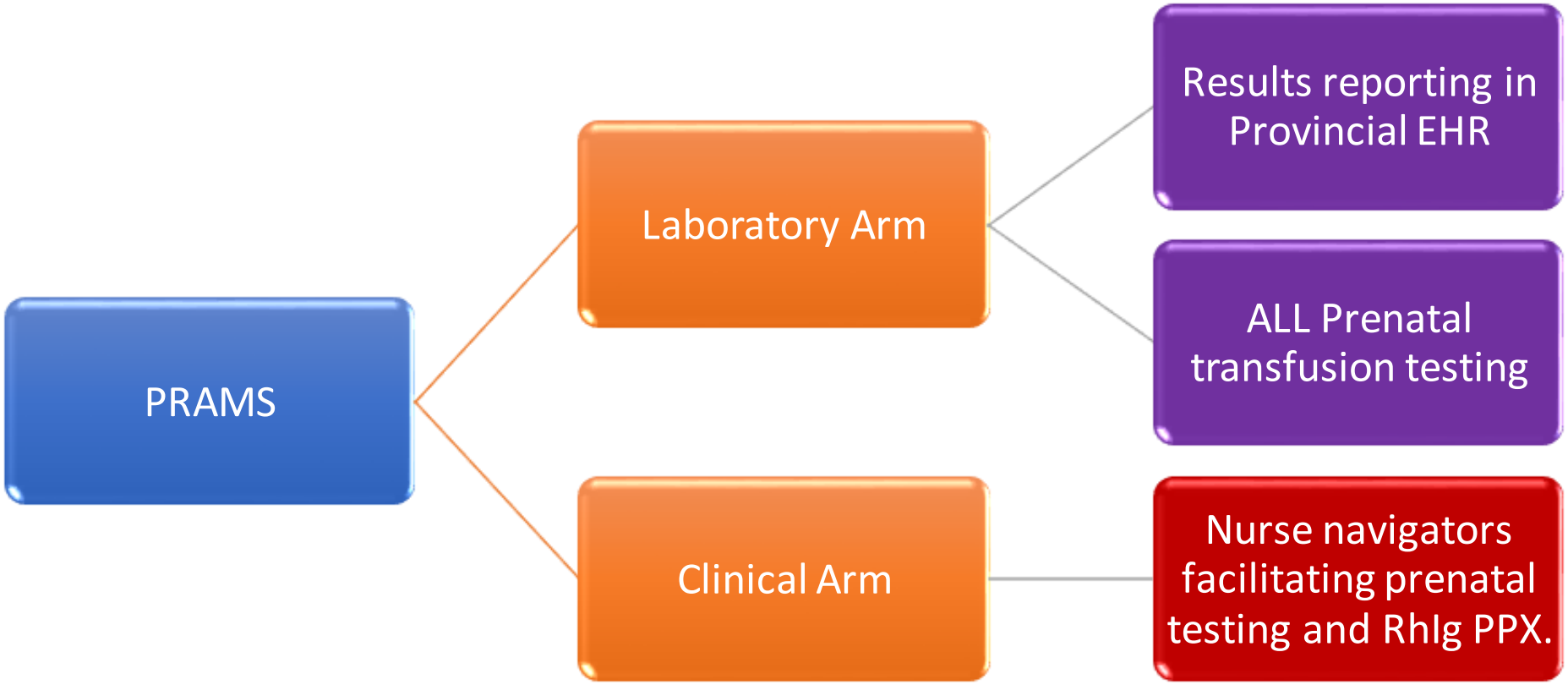
Why is the DAT positive

- RhIg can cross placenta and bind to Rh positive fetal red cells. But no adverse outcomes

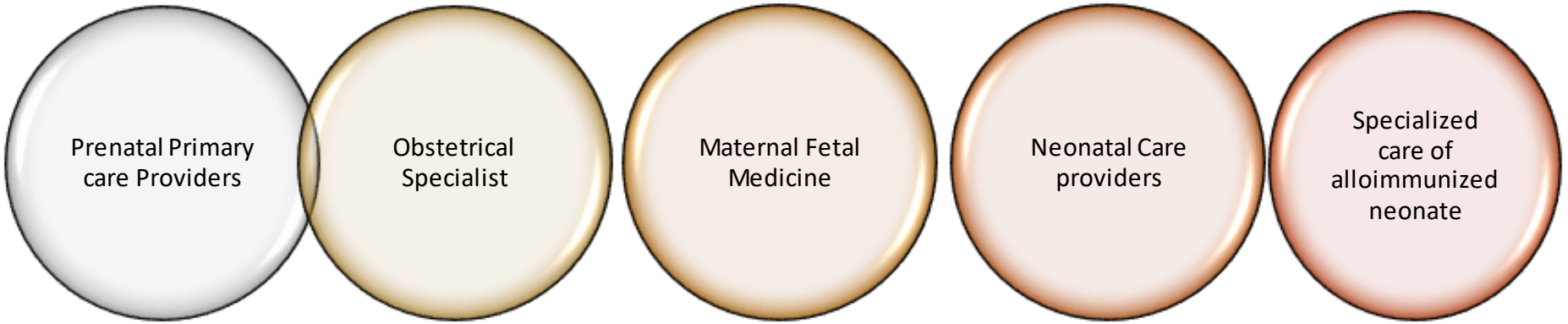
Cohen F, Zuelzer WW, Gustafson DC, Evans MM. Mechanisms of isoimmunization. I. The transplacental passage of fetal erythrocytes in homospecific pregnancies. Blood 1964;23:621–46. (Level III)
Lloyd LK, Miya F, Hebertson RM, Kochenour NK, Scott JR. Intrapartum fetomaternal bleeding in Rh-negative women. Obstet Gynecol 1980;5:285–8. (Level III)
Woodrow JC. Rh immunisation and its prevention. Ser Haematol 1970;3:1–151. (Level III) Sebring, E S, and H F Polesky. "Fetomaternal hemorrhage: incidence, risk factors, time of occurrence, and clinical effects." Transfusion vol. 30,4 (1990): 344-57. doi:10.1046/j.1537-2995.1990.30490273444.x

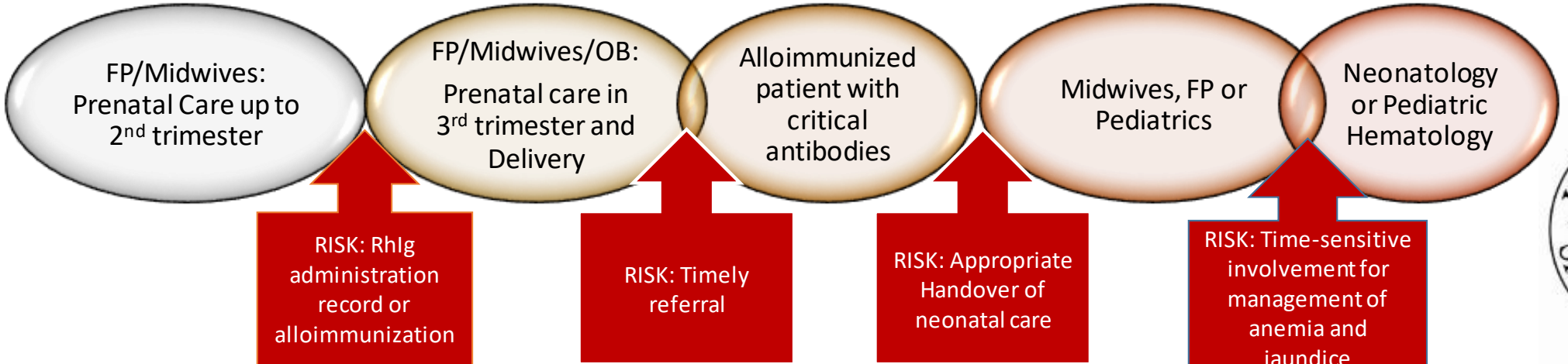
PRAMS Program

Prevention of Alloimmunization of mothers in Saskatchewan


PRAMS
Laboratory Arm
Clinical Arm
Results reporting in Provincial EHR
ALL Prenatal transfusion testing
Nurse navigators facilitating prenatal testing and RhIg PPX.

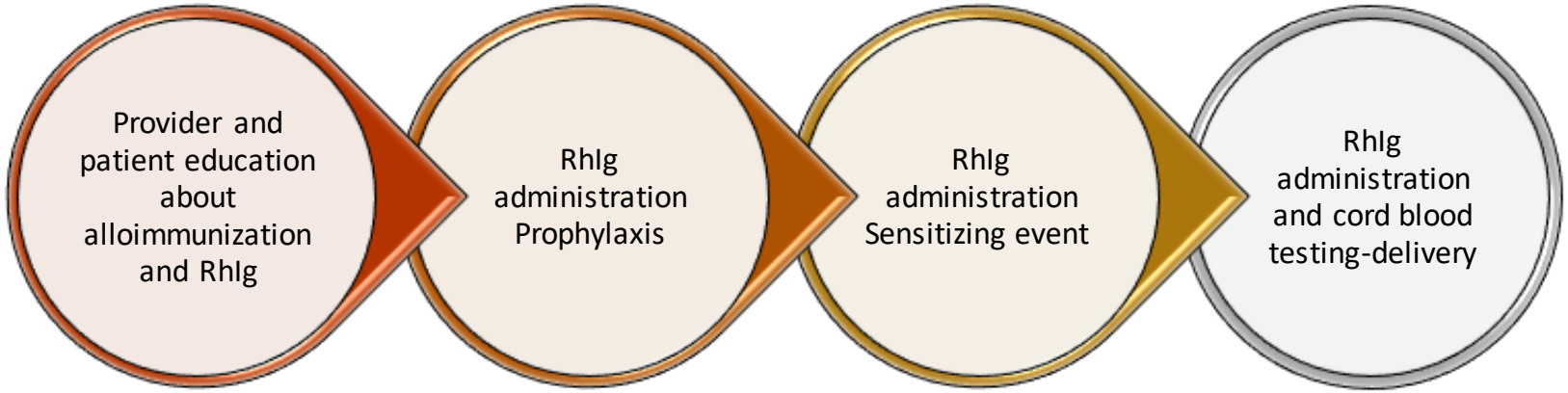
The chain of care providers for pregnancies



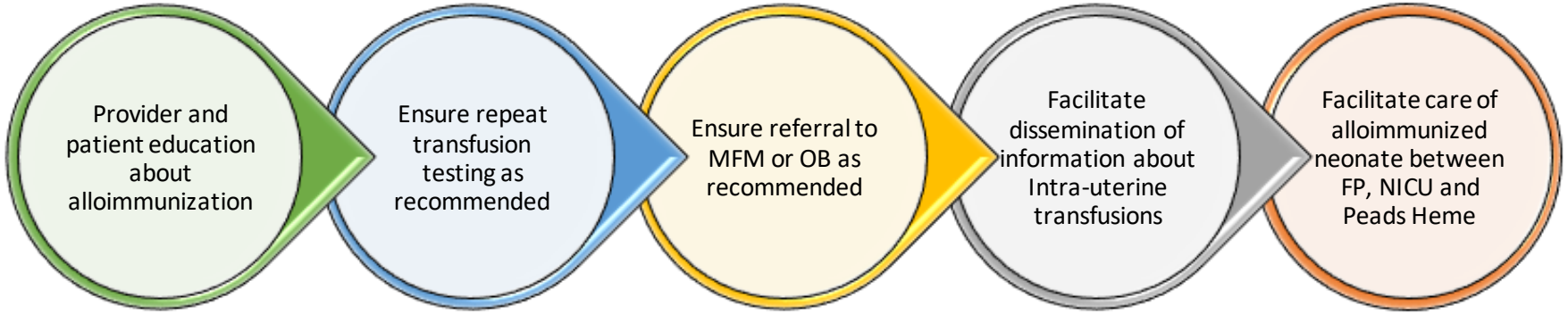


PRAMS nurse navigators ensure that the chain remains unbroken

RHD Negative Patient-Prevention of RhD alloimmunization



Alloimmunized Patient-Management of Alloimmunized pregnancy



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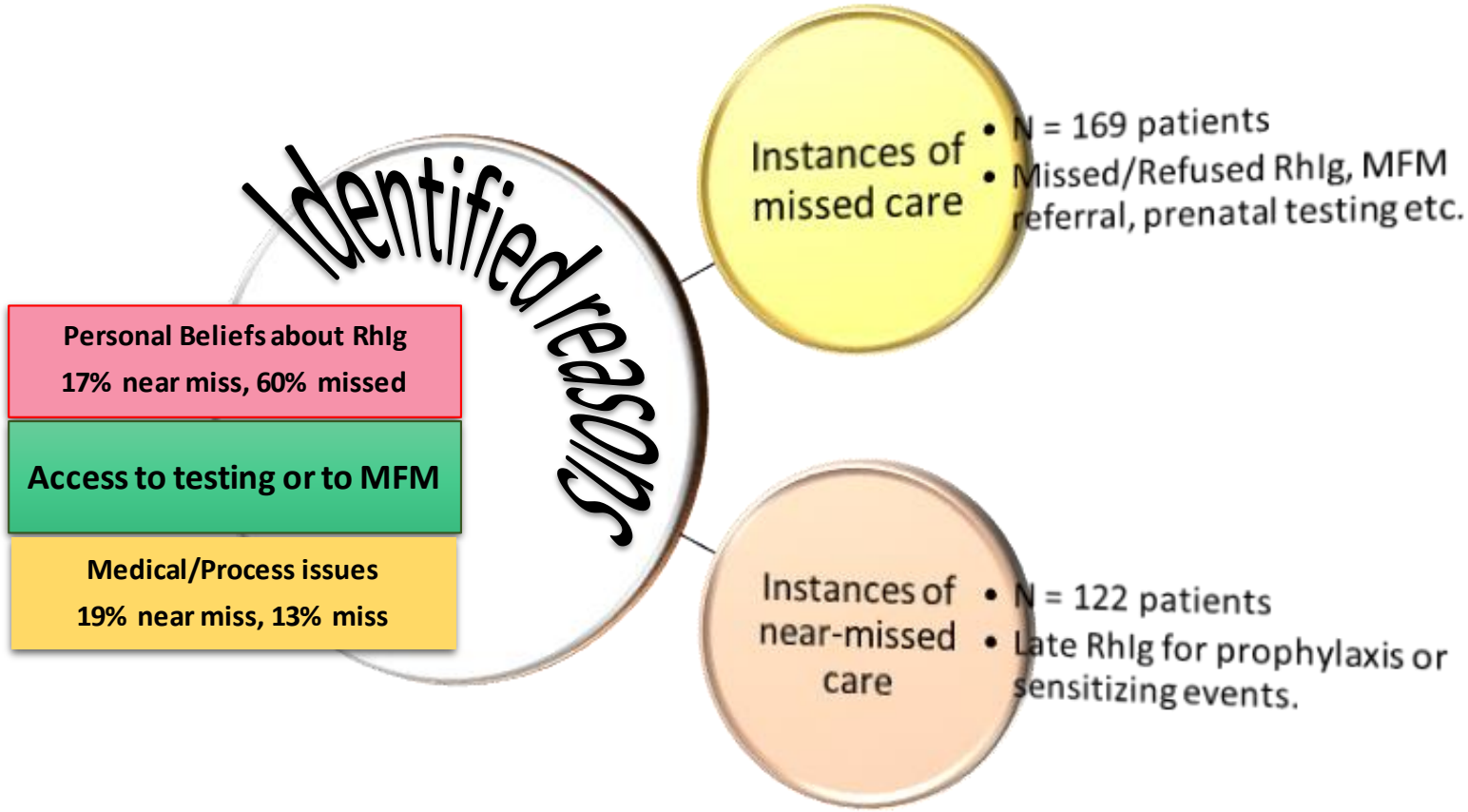
PRAMS-Clinical Arm Data since October 2021



Thank you for listening

Questions